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(54) Title: WSX RECEPTOR AND LIGANDS		
(57) Abstract The WSX receptor and antibodies which bind thereto (including agonist and neutralizing antibodies) are disclosed, including various uses therefor. Uses for WSX ligands (e.g., anti-WSX receptor agonist antibodies or OB protein) in hematopoiesis are also disclosed.		

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WSX RECEPTOR AND LIGANDS**CROSS REFERENCES**

This application is a continuation-in-part of co-pending U.S. Application Serial No. 08/667,197 filed June 20, 1996, which is a continuation-in-part of co-pending U.S. Application Serial No. 08/585,005 filed January 8, 1996, which applications are incorporated herein by reference and to which applications priority is claimed under 35 USC §120.

BACKGROUND OF THE INVENTION**Field of the Invention**

The present invention pertains generally to the WSX receptor and ligands and uses for these molecules.

10 Description of Related Art**A. HEMATOPOIESIS**

The process of blood cell formation whereby red and white blood cells are replaced through the division of cells located in the bone marrow is called hematopoiesis. For a review of hematopoiesis see Dexter and Spooncer (*Ann. Rev. Cell Biol.* 3:423-441 (1987)).

15 There are many different types of blood cells which belong to distinct cell lineages. Along each lineage, there are cells at different stages of maturation. Mature blood cells are specialized for different functions. For example, erythrocytes are involved in O₂ and CO₂ transport; T and B lymphocytes are involved in cell and antibody mediated immune responses, respectively; platelets are required for blood clotting; and the granulocytes and macrophages act as general scavengers and accessory cells. Granulocytes can be further divided into
20 basophils, eosinophils, neutrophils and mast cells.

Each of the various blood cell types arises from pluripotent or totipotent stem cells which are able to undergo self-renewal or give rise to progenitor cells or Colony Forming Units (CFU) that yield a more limited array of cell types. As stem cells progressively lose their ability to self-renew, they become increasingly lineage restricted. It has been shown that stem cells can develop into multipotent cells (called "CFC-Mix" by Dexter and Spooncer, *supra*). Some of the CFC-Mix cells can undergo renewal whereas others lead to lineage-restricted progenitors which eventually develop into mature myeloid cells (*e.g.*, neutrophils, megakaryocytes, macrophages and basophils). Similarly, pluripotent stem cells are able to give rise to PreB and PreT lymphoid cell lineages which differentiate into mature B and T lymphocytes, respectively. Progenitors are defined by their progeny, *e.g.*, granulocyte/macrophage colony-forming progenitor cells (GM-CFU) differentiate into neutrophils or
25 macrophages; primitive erythroid burst-forming units (BFU-E) differentiate into erythroid colony-forming units (CFU-E) which give rise to mature erythrocytes. Similarly, the Meg-CFU, Eos-CFU and Bas-CFU progenitors are able to differentiate into megakaryocytes, eosinophils and basophils, respectively.

Hematopoietic growth factors (reviewed in Andrea, *NEJM* 330(12):839-846 (1994)) have been shown to enhance growth and/or differentiation of blood cells via activation of receptors present on the surface of blood
35 progenitor cells of the bone marrow. While some of these growth factors stimulate proliferation of restricted lineages of blood cells, others enhance proliferation of multiple lineages of blood cells. For example, erythropoietin (EPO) supports the proliferation of erythroid cells, whereas interleukin-3 (IL-3) induces proliferation of erythroid and myeloid lineages and is therefore considered a multi-lineage factor.

In recent years, several hematopoietic growth factor receptors have been isolated. Due to their low abundance and their existence in both high-affinity and low-affinity forms, biochemical characterization of these receptors has been hampered.

Cytokine receptors frequently assemble into multi-subunit complexes. Sometimes, the α subunit of this complex is involved in binding the cognate growth factor and the β -subunit may contain an ability to transduce a signal to the cell. These receptors have been assigned to three subfamilies depending on the complexes formed. Subfamily 1 includes the receptors for erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), interleukin-4 (IL-4), interleukin-7 (IL-7), growth hormone (GH) and prolactin (PRL). Ligand binding to receptors belonging to this subfamily is thought to result in homodimerization of the receptor. Subfamily 2 includes receptors for IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-5 (IL-5), interleukin-6 (IL-6), leukemia inhibitory factor (LIF), oncostatin M (OSM) and ciliary neurotrophic factor (CNTF). Subfamily 2 receptors are heterodimers having an α -subunit for ligand binding and β -subunit (either the shared β -subunit of the IL-3, GM-CSF and IL-5 receptors or the gp130 subunit of the IL-6, LIF, OSM and CNTF receptors) for signal transduction. Subfamily 3 contains only the interleukin-2 (IL-2) receptor. The β and γ subunits of the IL-2 receptor complex are cytokine-receptor polypeptides which associate with the α -subunit of the unrelated Tac antigen.

B. OBESITY

Obesity is the most common nutritional disorder which, according to recent epidemiologic studies, affects about one third of all Americans 20 years of age or older. Kuczmarski *et al.*, *J. Am. Med. Assoc.* 272:205-211 (1994). Obesity is responsible for a variety of serious health problems, including cardiovascular disorders, type II diabetes, insulin-resistance, hypertension, hypertriglyceridemia, dyslipoproteinemia, and some forms of cancer. Pi-Sunyer, F., *Anns. Int. Med.* 119: 655-60 (1993); Colfitz, G., *Am. J. Clin. Nutr.* 55:503S-507S (1992). A single-gene mutation (the obesity or "*ob*" mutation) has been shown to result in obesity and type II diabetes in mice. Friedman, *Genomics* 11:1054-1062 (1991).

Zhang *et al.*, *Nature* 372:425-431 (1994) have recently reported the cloning and sequencing of the mouse *ob* gene and its human homologue, and suggested that the *ob* gene product, leptin or OB protein, may function as part of a signalling pathway from adipose tissue that acts to regulate the size of the body fat depot. Parabiosis experiments performed more than 20 years ago predicted that the genetically obese mouse containing two mutant copies of the *ob* gene (*ob/ob* mouse) does not produce a satiety factor which regulates its food intake, while the diabetic (*db/db*) mouse produces but does not respond to a satiety factor. Coleman and Hummal, *Am. J. Physiol.* 217:1298-1304 (1969); Coleman, *Diabetol* 9:294-98 (1973). Recent reports by three independent research teams have demonstrated that daily injections of recombinant OB protein inhibit food intake and reduce body weight and fat in grossly obese *ob/ob* mice but not in *db/db* mice (Pellemounter *et al.*, *Science* 269:540-43 (1995); Halaas *et al.*, *Science* 269:543-46 (1995); Campfield *et al.*, *Science* 269: 546-49 (1995)), suggesting that the OB protein is such a satiety factor as proposed in early cross-circulation studies.

Researchers suggest that at least one OB receptor is localized in the brain. The identification and expression cloning of a leptin receptor (OB-R) was reported by Tartaglia *et al.* *Cell* 83:1263-71 (1995). Various isoforms of a OB receptor are described by Cioffi *et al.* *Nature* 2:585-89 (1996). See, also, WO 96/08510.

The mouse *db* gene has recently been cloned (Lee *et al.* *Nature* 379:632 (1996) and Chen *et al.* *Cell* 84:491-495 (1996)). Previous data had suggested that the *db* gene encoded the receptor for the obese (*ob*) gene product, leptin (Coleman *et al.*, *Diabetologia* 9:294-8 (1973) and Coleman *et al.*, *Diabetologia* 14:141-8 (1978)). It has been very recently confirmed that the *db/db* mouse results from a truncated splice variant of the OB receptor which likely renders the receptor defective in signal transduction (Lee *et al.*, *Nature* 379:632 (1996) and Chen *et al.*, *Cell* 84: 491-495 (1996)).

SUMMARY OF THE INVENTION

The invention herein is concerned with the WSX cytokine receptor and a soluble form of the receptor which is the WSX receptor extracellular domain (ECD). The WSX receptor polypeptides are optionally conjugated with, or fused to, molecules which increase the serum half-lives thereof and can be formulated as pharmaceutical compositions comprising the polypeptide and a physiologically acceptable carrier.

In certain embodiments, the WSX receptor ECD may be used as an antagonist insofar as it may bind to WSX ligand and thereby reduce activation of endogenous WSX receptor. This may be useful in conditions characterized by excess levels of WSX ligand and/or excess WSX receptor activation in a mammal. WSX receptor ECD may, for example, be used to treat metabolic disorders (*e.g.*, anorexia or steroid-induced truncal obesity), stem cell tumors and other tumors which express WSX receptor.

Pharmaceutical compositions of the WSX receptor ECD may further include a WSX ligand. Such dual compositions may be beneficial where it is therapeutically useful to prolong the half-life of WSX ligand and/or activate endogenous WSX receptor directly as a heterotrimeric complex.

The invention also relates to chimeric WSX receptor molecules, such as WSX receptor immunoadhesins (having long half-lives in the serum of a patient treated therewith) and epitope tagged WSX receptor. Immunoadhesins may be employed as WSX receptor antagonists in conditions or disorders in which neutralization of WSX receptor biological activity may be beneficial. Bispecific immunoadhesins (combining a WSX receptor ECD with a domain of another cytokine receptor) may form high affinity binding complexes for WSX ligand.

The invention further provides methods for identifying a molecule which binds to and/or activates the WSX receptor. This is useful for discovering molecules (such as peptides, antibodies, and small molecules) which are agonists or antagonists of the WSX receptor. Such methods generally involve exposing an immobilized WSX receptor to a molecule suspected of binding thereto and determining binding of the molecule to the immobilized WSX receptor and/or evaluating whether or not the molecule activates (or blocks activation of) the WSX receptor. In order to identify such WSX ligands, the WSX receptor may be expressed on the surface of a cell and used to screen libraries of synthetic compounds and naturally occurring compounds (*e.g.*, endogenous sources of such naturally occurring compounds, such as serum). The WSX receptor can also be used as a diagnostic tool for measuring serum levels of endogenous WSX ligand.

In a further embodiment, a method for purifying a molecule which binds to the WSX receptor is provided. This can be used in the commercial production and purification of therapeutically active molecules which bind to this receptor. In the method, the molecule of interest (generally a composition comprising one or more contaminants) is adsorbed to immobilized WSX receptor (*e.g.*, WSX receptor immunoadhesin immobilized on a protein A column). The contaminants, by virtue of their inability to bind to the WSX receptor, will

generally flow through the column. Accordingly, it is then possible to recover the molecule of interest from the column by changing the elution conditions, such that the molecule no longer binds to the immobilized receptor.

In further embodiments, the invention provides antibodies that specifically bind to the WSX receptor. Preferred antibodies are monoclonal antibodies which are non-immunogenic in a human and bind to an epitope
5 in the extracellular domain of the receptor. Preferred antibodies bind the WSX receptor with an affinity of at least about 10^6 L/mole, more preferably 10^7 L/mole.

Antibodies which bind to the WSX receptor may optionally be fused to a heterologous polypeptide and the antibody or fusion thereof may be used to isolate and purify WSX receptor from a source of the receptor.

In a further aspect, the invention provides a method for detecting the WSX receptor *in vitro* or *in vivo*
10 comprising contacting the antibody with a sample suspected of containing the receptor and detecting if binding has occurred. Based on the observation herein that CD34+ cells possess WSX receptor, use of WSX antibodies for identification and/or enrichment of stem cell populations (in a similar manner to that in which CD34 antibodies are presently used) is envisaged.

For certain applications, it is desirable to have an agonist antibody which can be screened for as
15 described herein. Such agonist antibodies are useful for activating the WSX receptor for *in vitro* uses whereby enhancement of proliferation and/or differentiation of a cell comprising the receptor is desired. Furthermore, these antibodies may be used to treat conditions in which an effective amount of WSX receptor activation leads to a therapeutic benefit in the mammal treated therewith. For example, the agonist antibody can be used to enhance survival, proliferation and/or differentiation of a cell comprising the WSX receptor. In particular, agonist
20 antibodies and other WSX ligands may be used to stimulate proliferation of stem cells/progenitor cells either *in vitro* or *in vivo*. Other potential therapeutic applications include the use of agonist antibodies to treat metabolic disorders (such as obesity and diabetes) and to promote kidney, liver or lung growth and/or repair (e.g., in renal failure).

For therapeutic applications it is desirable to prepare a composition comprising the agonist antibody
25 and a physiologically acceptable carrier. Optionally, such a composition may further comprise one or more cytokines.

In other embodiments, the antibody is a neutralizing antibody. Such molecules can be used to treat conditions characterized by unwanted or excessive activation of the WSX receptor.

In addition to the above, the invention provides isolated nucleic acid molecules, expression vectors and
30 host cells encoding the WSX receptor which can be used in the recombinant production of WSX receptor as described herein. The isolated nucleic acid molecules and vectors are also useful for gene therapy applications to treat patients with WSX receptor defects and/or to increase responsiveness of cells to WSX ligand.

This application also relates to agonist antibodies which specifically bind to the WSX receptor and mimic one or more biological activities of naturally occurring WSX ligand, OB protein. Preferred antibodies are
35 those with a strong binding affinity for human WSX receptor (e.g. having a K_d of no more than about 1×10^8 M; and preferably no more than about 1×10^9 M). In preferred embodiments, the agonist antibody binds to both human and murine WSX receptor.

Antibodies with defined agonistic activity in a bioassay, the KIRA ELISA, are disclosed herein. Preferred antibodies have an IC₅₀ in the KIRA ELISA of about 0.5 µg/ml or less, preferably about 0.2 µg/ml or less, and most preferably about 0.1 µg/ml or less.

The agonist antibodies of interest herein may have one or more of the biological characteristics of antibody 2D7, 1G4, 1E11 or 1C11 (see Example 13) or clones 3, 4, or 17 (see Example 14). For example, the antibody may bind to the epitope bound by any one of these antibodies, and/or may have some or all of the hypervariable region residues of these antibodies.

The agonist antibody may be one which decreases body weight and/or fat-depot weight and/or food intake in an obese mammal (e.g. in an *ob/ob* mouse). The preferred agonist antibody is one which exerts an adipose-reducing effect in an obese mammal (e.g. an *ob/ob* mouse) which is in excess of that induced by a reduction in food intake (Levin *et al. Proc. Natl. Acad. Sci. USA* 93:1726-1730 (1996)).

The agonist antibody may also have the property of inducing differentiation and/or proliferation and/or survival of hematopoietic progenitor cells. For example, the agonist antibody may induce lymphopoiesis, erythropoiesis and/or myelopoiesis.

The invention further provides a composition comprising the agonist antibody and a physiologically acceptable carrier. The composition for therapeutic use is sterile and may be lyophilized. For use in hematopoiesis, for example, the composition may further comprise a cytokine.

In another aspect, the invention provides a method for activating the WSX receptor which comprises exposing the WSX receptor to an amount of an agonist anti-WSX receptor antibody which is effective for activating the WSX receptor. The invention further provides a method for enhancing proliferation and/or differentiation of a cell which expresses the WSX receptor at its cell surface comprising exposing the cell to an amount of exogenous agonist anti-WSX receptor antibody which is effective for enhancing proliferation and/or differentiation of the cell. In another embodiment, the invention provides a method for decreasing body weight and/or fat-depot weight and/or food intake in an obese mammal (e.g. a human) comprising administering an effective amount of the agonist antibody to the mammal. Also, the invention provides a method for treating the medical sequelae of obesity in a mammal, such as, e.g., arteriosclerosis, Type II diabetes, polycystic ovarian disease, cardiovascular diseases, osteoarthritis, dermatological disorders, hypertension, insulin resistance, hypercholesterolemia, hypertriglyceridemia, cancer and cholelithiasis, comprising administering an effective amount of an agonist anti-WSX receptor antibody to the mammal. The mammal to be treated may be one diagnosed with any one or more of these diseases, or may be predisposed to these diseases.

In another aspect, the present invention pertains to the discovery herein that WSX ligands, such as obesity (OB) protein, play a role in hematopoiesis via signalling through the WSX receptor. The role of the WSX receptor-ligand signalling pathway appears to be at the level of the early hematopoietic precursor as is evident by the ability of OB protein to simulate myelopoiesis, erythropoiesis (e.g. splenic erythropoiesis) and most dramatically, lymphopoiesis. Accordingly, WSX ligands can be used to stimulate proliferation and/or differentiation and/or survival of hematopoietic progenitor cells either *in vitro* or *in vivo* (e.g. for treating hematopoietic diseases or disorders).

Thus, the invention provides a method for stimulating proliferation and/or differentiation of a cell which expresses the WSX receptor (especially the WSX receptor variant 13.2, which is demonstrated herein to have

the capacity to transmit a proliferative signal) at its cell surface comprising the step of contacting the WSX receptor with an amount of WSX ligand which is effective for stimulating proliferation and/or OB protein differentiation of the cell. In preferred embodiments, the cell which is exposed to the WSX ligand is a hematopoietic precursor, *e.g.* a CD34+ cell. The WSX ligand may be OB protein or an agonist antibody which
5 binds to the WSX receptor. For *in vivo* use, the WSX ligand of choice may be a long half-life derivative of an OB protein, such as OB-immunoglobulin chimera and/or OB protein modified with a nonproteinaceous polymer, such as polyethylene glycol (PEG). The method contemplated herein may lead to an increase in the proliferation and/or differentiation of lymphoid, myeloid and/or erythroid blood cell lineages and encompasses both *in vitro* and *in vivo* methods. For *in vitro* uses, the cell possessing the WSX receptor may be present in cell culture. As
10 to *in vivo* methods, the cell may be present in a mammal, especially a human (*e.g.* one who is suffering from decreased blood levels and who could benefit from an increase in various blood cells). Potential patients include those who have undergone chemo- or radiation therapy, or bone marrow transplantation therapy. Thus, the invention provides a method for repopulating blood cells (*e.g.* erythroid, myeloid and/or lymphoid blood cells) in a mammal comprising administering to the mammal a therapeutically effective amount of a WSX ligand.

15 Mammals which may benefit from an enhancement of lymphopoiesis include those predisposed to, or suffering from, any one or more of the following exemplary conditions: lymphocytopenia; lymphorrhea; lymphostasis; immunodeficiency (*e.g.* HIV and AIDS); infections (including, for example, opportunistic infections and tuberculosis (TB)); lupus; and other disorders characterized by lymphocyte deficiency. An effective amount of the WSX ligand can be used in a method of immunopotentialization or to improve immune
20 function in a mammal.

On the other hand, WSX receptor or WSX ligand antagonists (such as WSX receptor ECD or immunoadhesin, and WSX receptor or OB protein neutralizing antibodies) may be used in the treatment of those disorders wherein unacceptable lymphocyte levels are present in the mammal, particularly where this is caused by excessive activation of the WSX receptor. Examples of conditions in which administration of such an
25 antagonist may be beneficial include: neoplastic disorders (such as Hodgkin's disease; lymphosarcoma; lymphoblastoma; lymphocytic leukemia; and lymphoma) and lymphocytosis.

Diseases or disorders in which an increase in erythropoiesis may be beneficial include, but are not limited to: erythrocytopenia; erythrodegenerative disorders; erythroblastopenia; leukoerythroblastosis; erythroclasis; thalassemia; and anemia (*e.g.* hemolytic anemia, such as acquired, autoimmune, or
30 microangiopathic hemolytic anemia; aplastic anemia; congenital anemia, *e.g.*, congenital dyserythropoietic anemia, congenital hemolytic anemia or congenital hypoplastic anemia; dyserythropoietic anemia; Facioni's anemia; genetic anemia; hemorrhagic anemia; hyperchromic or hypochromic anemia; nutritional, hypoferric, or iron deficiency anemia; hypoplastic anemia; infectious anemia; lead anemia; local anemia; macrocytic or microcytic anemia; malignant or pernicious anemia; megaloblastic anemia; molecular anemia; normocytic anemia;
35 physiologic anemia; traumatic or posthemorrhagic anemia; refractory anemia; radiation anemia; sickle cell anemia; splenic anemia; and toxic anemia).

Conversely, WSX receptor or WSX ligand antagonists may be used to treat those conditions in which excessive erythrocyte levels are present in a mammal, *e.g.* in neoplastic disorders such as erythroleukemia; erythroblastosis; and erythrocythemia or polycythemia.

An increase in myelopoiesis may be beneficial in any of the above-mentioned diseases or disorders as well as the following exemplary conditions: myelofibrosis; thrombocytopenia; hypoplasia; disseminated intravascular coagulation (DIC); immune (autoimmune) thrombocytopenic purpura (ITP); HIV induced ITP; myelodysplasia; thrombocytotic diseases and thrombocytosis.

5 Antagonists of the WSX receptor-WSX ligand interaction may also be used to treat myeloid cell-related conditions such as malignancies (e.g. myelosarcoma, myeloblastoma, myeloma, myeloleukemia and myelocytomatosis); myeloblastosis; myelocytosis; and myelosis.

The method may further involve the step of exposing hematopoietic cells (whether they be in cell culture or in a mammal) to one or more other cytokines (e.g. lineage-specific cytokines) and this may lead to a synergistic enhancement of the proliferation and/or differentiation of the cells. Exemplary cytokines include
 10 thrombopoietin (TPO); erythropoietin (EPO); macrophage-colony stimulating factor (M-CSF); granulocyte-macrophage-CSF (GM-CSF); granulocyte-CSF (G-CSF); interleukin-1 (IL-1); IL-1 α ; IL-2; IL-3; IL-4; IL-5; IL-6; IL-7; IL-8; IL-9; IL-11; IL-10; IL-12; leukemia inhibitory factor (LIF) or kit ligand (KL). In this embodiment, exposure to the cytokine may proceed, occur simultaneously with, or follow, exposure to the WSX ligand.
 15 Preferably, the WSX ligand and one or more further cytokines are administered simultaneously to the patient (where the method is an *in vivo* one) and, optionally, are combined to form a pharmaceutical composition.

For use in the above methods, the invention also provides an article of manufacture, comprising: a container; a label on the container; and a composition comprising an active agent within the container; wherein the composition is effective for enhancing proliferation and/or differentiation of cells comprising the WSX
 20 receptor in a mammal, the label on the container indicates that the composition can be used for enhancing proliferation and/or differentiation of those cells and the active agent in the composition is a WSX ligand. Optionally, the article of manufacture includes one or more further containers which hold further cytokine(s) in a packaged combination with the container holding the WSX ligand.

In another embodiment, an effective amount of the WSX ligand may be used to improve engraftment
 25 in bone marrow transplantation or to stimulate mobilization of hematopoietic stem cells in a mammal prior to harvesting hematopoietic progenitors from the peripheral blood thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-H together depict the double stranded nucleotide (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) encoding full length human WSX receptor variant 13.2. Nucleotides are numbered
 30 at the beginning of the sense strand. Amino acid residues are numbered at the beginning of the amino acid sequence. Restriction enzyme sites are depicted above the nucleotide sequence.

Figs. 2A-B together depict an amino acid sequence alignment of full length human WSX receptor variants 6.4 (SEQ ID NO:3), 12.1 (SEQ ID NO:4) and 13.2, respectively. Homologous residues are boxed. WSX receptor variants 6.4, 12.1 and 13.2 are native sequence human WSX receptor variants which, without
 35 being bound to any one theory, appear to be generated by alternate splicing of WSX receptor mRNA. The putative signal peptide, transmembrane, Box 1, Box 2, and Box 3 domains are indicated. The extracellular and cytoplasmic domains are amino- and carboxy-terminal, respectively, to the transmembrane domain. The Box 1-3 domains shown correspond to the box 1-3 motifs described in Baumann *et al.*, *Mol. Cell. Biol.* 14(1):138-146 (1994).

Figs. 3A-E together depict an alignment of the nucleotide sequences encoding human WSX receptor variants 6.4 (SEQ ID NO:5), 12.1 (SEQ ID NO:6) and 13.2, respectively.

Figs. 4A-B depict an alignment of the full length human WSX receptor variant 13.2 amino acid sequence (top) with that of partial murine WSX receptor extracellular domain sequence (bottom) (SEQ ID NO:7) obtained as described in Example 7. The putative murine signal peptide is marked with an arrow.

Figs. 5A-F represent an alignment of the nucleotide sequences encoding human WSX receptor variant 13.2 (bottom) and partial murine WSX receptor extracellular domain (top) (SEQ ID NO:8), respectively.

Fig. 6 is a bar graph depicting results of the thymidine incorporation assay described in Example 5. ³H-thymidine incorporation (counts per minute, CPM) in parental Baf3 cells or Baf3 cells electroporated with GH/WSX variant 13.2 chimera in the presence of varying concentrations of human growth hormone (GH) is shown.

Fig. 7 shows the human and murine oligonucleotides (SEQ ID NOS:9-38, respectively) used for the antisense experiment described in Example 8.

Figs. 8 and 9 show thymidine incorporation assays in Baf-3 cells. For these assays, cells were deprived of IL-3 for 16-18 hours (in RPMI 1640 supplemented with 10% fetal calf serum (FCS)). Cells were washed in serum free RPMI 1640 and plated at 50,000 cells per well in 0.2 mls of serum free RPMI 1640 supplemented with the indicated concentration of human GH or human OB protein. Cells were stimulated for 24 hours and thymidine incorporation was determined as described (Zeigler *et al. Blood* 84:2422-2430 (1994)). Assays were performed in triplicate and the results were confirmed in three independent experiments.

In Fig. 8, GH receptor-WSX receptor variant 12.1 or 13.2 chimeric proteins were expressed in Baf-3 cells as described in Example 5. These transfected cells and the parental Baf-3 line were stimulated with hGH and the incorporation of titrated thymidine determined.

In Fig. 9, Baf-3 cells were stably transfected with WSX receptor variant 13.2. Thymidine incorporation was then determined in these cell lines following stimulation with human OB protein.

In Figs. 10A-C, murine fetal liver AA4⁺Sca⁺Kit⁺ (fASK) stem cells were cultured in suspension culture or methylcellulose. In Fig. 10A, fASK cells were cultured in suspension culture containing serum with kit ligand (KL) or kit ligand and OB protein. Cell counts and cytopsin analyses were performed 7 days later. In Fig. 10B, fASK cells were seeded into methylcellulose under either myeloid or lymphoid conditions as described in Example 10. Colony counts were performed 14 days later. For colonies produced under lymphoid conditions, FACS analysis demonstrated the vast majority of cells to be B220 positive. In Fig. 10C, fASK cells were seeded into methylcellulose containing kit ligand. To this base media, erythropoietin (EPO) or erythropoietin and OB protein were then added. The resultant colonies were counted 14 days later. FACS analysis demonstrated approximately 95% of these colonies to be TER 119 positive. All assays were performed in triplicate and confirmed in at least three independent experiments.

Fig. 11 illustrates methylcellulose assays to determine the colony forming potential of *db/db*, *ob/ob* and the corresponding wild-type marrow. 100,000 bone marrow cells were seeded into methylcellulose and the resultant colonies counted after 14 days. Assays were performed using both myeloid and lymphoid conditions. Assays were performed in triplicate and the experiments were repeated a minimum of 3 times.

Figs. 12A-B show bone marrow cellular profiles in wild-type misty gray homozygotes, misty gray/*db* heterozygotes, and homozygote *db/db* mice. Overall cellularity in the *db/db* marrow was unchanged compared to controls. Fig. 12A shows cellular profiles determined using anti-B220, anti-CD43, and anti-TER119 antibodies. Fig. 12B shows cellular profiles of the spleens from the above groups.

5 Figs. 13A-C are an analysis of peripheral blood in *db/db* homozygotes, *db/db* misty gray heterozygotes and misty gray homozygotes. 40 microliters of peripheral blood was taken via orbital bleed and analyzed on a Serrono Baker system 9018. All areas described by the boxes represent the mean \pm one standard deviation of the two parameters.

10 Fig. 14 is a comparison of peripheral lymphocyte counts and blood glucose level. Five groups of animals, misty-gray, misty-gray/*db*, *db/db*, interferon α -transgenic, and glucokinase transgenic heterozygote mice (gLKa) were sampled via retro-orbital bleed. Blood glucose levels in these mice were determined. All areas described by the boxes represent the mean \pm standard deviation of the two parameters.

15 In Figs. 15A-C, misty gray homozygotes, *db*/misty gray heterozygotes, and homozygous *db/db* mice were subjected to sub-lethal irradiation and the recovery kinetics of the peripheral blood was determined via retro-orbital bleeds.

Figs. 16A-16Q together show the nucleotide sequence (SEQ ID NO:46) and the amino acid sequence (SEQ ID NO: 47) of the human OB-immunoglobulin chimera in the plasmid described in of Example 11.

20 Fig. 17 shows binding of anti-WSX receptor agonist antibodies to human WSX receptor. The anti-WSX receptor agonist antibodies (2D7 and 1G4) produced as described in Example 13 and an IgG isotope control were evaluated for their ability to bind to human WSX receptor by capture ELISA.

Fig. 18 shows the activity of mAbs 2D7 and 1G4 as well as OB protein in the KIRA ELISA (see Example 13). Absorbance at 490nm versus concentration of antibody or ligand in this assay is shown.

25 Fig. 19 depicts binding of anti-WSX receptor agonist antibodies to murine WSX receptor. The anti-WSX receptor agonist antibodies (2D7 and 1G4) and an IgG isotope control were evaluated for their ability to bind to murine WSX receptor by capture ELISA.

30 Figs. 20A-B show the results of epitope mapping of the agonist anti-WSX receptor antibodies produced as described in Example 13. Fig. 20A shows blocking ability of anti-WSX receptor antibodies on Epitope A using biotinylated 2D7. Fig. 20B shows blocking ability of anti-WSX receptor antibodies on Epitope B using biotinylated 1C11. Based on the competitive binding ELISA, 2D7 bound a different epitope from 1E11, 1C11 and 1G4.

Fig. 21 depicts an alignment of the amino acid sequences of full length human WSX receptor variant 6.4 (hWSXR) (SEQ ID NO:3) and murine WSX receptor (mWSXR) (SEQ ID NO:51).

Fig. 22 is a standard curve for human OB protein in the KIRA ELISA, which illustrates schematically inside the graph WSX receptor KIRA ELISA panning with scFv phage as described in Example 14.

35 Fig. 23 shows the activity of clone # 3, #4 and # 17 scFv phage from Example 14 and anti-HER2 scFv phage control in the KIRA ELISA. Absorbance versus phage titer is shown.

Fig. 24 shows the activity of clone # 3, #4 and # 17 scFv from Example 14, anti-HER2 scFv control (Her2 clone) and OB protein in the KIRA ELISA. Absorbance versus antibody concentration is shown.

Fig. 25 aligns the amino acid sequences of agonist antibody clone #3 (3.scFv) (SEQ ID NO:48), clone #4 (4.scFv) (SEQ ID NO:49) and clone #17 (17.scFv) (SEQ ID NO:50) obtained as described in Example 14. Complementarity determining region (CDR) residues according to Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991) are underlined and hypervariable loop residues (Chothia *et al.*, *Nature* 342:8767 (1989)) are in italics.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

The terms "WSX receptor" or "WSX receptor polypeptide" when used herein encompass native sequence WSX receptor; WSX receptor variants; WSX extracellular domain; and chimeric WSX receptor (each of which is defined herein). Optionally, the WSX receptor is not associated with native glycosylation. "Native glycosylation" refers to the carbohydrate moieties which are covalently attached to WSX receptor when it is produced in the mammalian cell from which it is derived in nature. Accordingly, human WSX receptor produced in a non-human cell is an example of a WSX receptor which is "not associated with native glycosylation". Sometimes, the WSX receptor is unglycosylated (*e.g.*, as a result of being produced recombinantly in a prokaryote).

"WSX ligand" is a molecule which binds to and activates native sequence WSX receptor (especially WSX receptor variant 13.2). The ability of a molecule to bind to WSX receptor can be determined by the ability of a putative WSX ligand to bind to WSX receptor immunoadhesin (see Example 2) coated on an assay plate, for example. The thymidine incorporation assay provides a means for screening for WSX ligands which activate the WSX receptor. Exemplary WSX ligands include anti-WSX receptor agonist antibodies and OB protein (*e.g.*, described in Zhang *et al.* *Nature* 372:425-431 (1994)).

The terms "OB protein" and "OB" are used interchangeably herein and refer to native sequence OB proteins (also known as "leptins") and their functional derivatives.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (*e.g.*, WSX receptor or OB protein) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "native sequence WSX receptor" specifically encompasses naturally-occurring truncated forms of the WSX receptor, naturally-occurring variant forms (*e.g.*, alternatively spliced forms such as human WSX receptor variants 6.4, 12.1 and 13.2 described herein) and naturally-occurring allelic variants of the WSX receptor. The preferred native sequence WSX receptor is a mature native sequence human WSX receptor, such as human WSX receptor variant 6.4, human WSX receptor variant 12.1 or human WSX receptor variant 13.2 (each shown in Figs. 2A-B). Most preferred is mature human WSX receptor variant 13.2.

The term "native sequence OB protein" includes those OB proteins from any animal species (*e.g.* human, murine, rabbit, cat, cow, sheep, chicken, porcine, equine, etc.) as occurring in nature. The definition specifically includes variants with or without a glutamine at amino acid position 49, using the amino acid

numbering of Zhang *et al.*, *supra*. The term "native sequence OB protein" includes the native proteins with or without the initiating N-terminal methionine (Met), and with or without the native signal sequence, either in monomeric or in dimeric form. The native sequence human and murine OB proteins known in the art are 167 amino acids long, contain two conserved cysteines, and have the features of a secreted protein. The protein is largely hydrophilic, and the predicted signal sequence cleavage site is at position 21, using the amino acid numbering of Zhang *et al.*, *supra*. The overall sequence homology of the human and murine sequences is about 84%. The two proteins show a more extensive identity in the N-terminal region of the mature protein, with only four conservative and three non-conservative substitutions among the residues between the signal sequence cleavage site and the conserved Cys at position 117. The molecular weight of OB protein is about 16 kD in a monomeric form.

The "WSX receptor extracellular domain" (ECD) is a form of the WSX receptor which is essentially free of the transmembrane and cytoplasmic domains of WSX receptor, *i.e.*, has less than 1% of such domains, preferably 0.5 to 0% of such domains, and more preferably 0.1 to 0% of such domains. Ordinarily, the WSX receptor ECD will have an amino acid sequence having at least about 95% amino acid sequence identity with the amino acid sequence of the ECD of WSX receptor indicated in Figs. 2A-B for human WSX receptor variants 6.4, 12.1 and 13.2, preferably at least about 98%, more preferably at least about 99% amino acid sequence identity, and thus includes WSX receptor variants as defined below.

A "variant" polypeptide means a biologically active polypeptide as defined below having less than 100% sequence identity with a native sequence polypeptide (*e.g.*, WSX receptor having the deduced amino acid sequence shown in Figs. 1A-H for human WSX receptor variant 13.2). Such variants include polypeptides wherein one or more amino acid residues are added at the N- or C-terminus of, or within, the native sequence; from about one to thirty amino acid residues are deleted, and optionally substituted by one or more amino acid residues; and derivatives of the above polypeptides, wherein an amino acid residue has been covalently modified so that the resulting product has a non-naturally occurring amino acid. Ordinarily, a biologically active WSX receptor variant will have an amino acid sequence having at least about 90% amino acid sequence identity with human WSX receptor variant 13.2 shown in Figs. 1A-H, preferably at least about 95%, more preferably at least about 99%. Ordinarily, a biologically active OB protein variant will have an amino acid sequence having at least about 90% amino acid sequence identity with a native sequence OB protein, preferably at least about 95%, more preferably at least about 99%.

A "chimeric" OB protein or WSX receptor is a polypeptide comprising OB protein or full-length WSX receptor or one or more domains thereof (*e.g.*, the extracellular domain of the WSX receptor) fused or bonded to heterologous polypeptide. The chimeric WSX receptor will generally share at least one biological property in common with human WSX receptor variant 13.2. The chimeric OB protein will generally share at least one biological property in common with a native sequence OB protein. Examples of chimeric polypeptides include immunoadhesins and epitope tagged polypeptides.

The term "WSX immunoadhesin" is used interchangeably with the expression "WSX receptor-immunoglobulin chimera" and refers to a chimeric molecule that combines a portion of the WSX receptor (generally the extracellular domain thereof) with an immunoglobulin sequence. Likewise, an "OB protein immunoadhesin" or "OB-immunoglobulin chimera" refers to a chimeric molecule which combines OB protein

(or a portion thereof) with an immunoglobulin sequence. The immunoglobulin sequence preferably, but not necessarily, is an immunoglobulin constant domain. The immunoglobulin moiety in the chimeras of the present invention may be obtained from IgG1, IgG2, IgG3 or IgG4 subtypes, IgA, IgE, IgD or IgM, but preferably IgG1 or IgG3.

5 The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising WSX receptor or OB protein fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody thereagainst can be made, yet is short enough such that it does not interfere with biological activity of the WSX receptor or OB protein. The tag polypeptide preferably also is fairly unique so that the antibody thereagainst does not substantially cross-react with other epitopes. Suitable tag polypeptides
10 generally have at least six amino acid residues and usually between about 8-50 amino acid residues (preferably between about 9-30 residues).

 "Isolated" WSX receptor (or OB protein) means WSX receptor (or OB protein) that has been purified from a WSX receptor (or OB protein) source or has been prepared by recombinant or synthetic methods and is sufficiently free of other peptides or proteins (1) to obtain at least 15 and preferably 20 amino acid residues of
15 the N-terminal or of an internal amino acid sequence by using a spinning cup sequenator or the best commercially available amino acid sequenator marketed or as modified by published methods as of the filing date of this application, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Homogeneity here means less than about 5% contamination with other source proteins.

20 "Essentially pure" protein means a composition comprising at least about 90% by weight of the protein, based on total weight of the composition, preferably at least about 95% by weight. "Essentially homogeneous" protein means a composition comprising at least about 99% by weight of protein, based on total weight of the composition.

 "Biological property" when used in conjunction with either "WSX receptor" or "isolated WSX receptor"
25 means having an effector or antigenic function or activity that is directly or indirectly caused or performed by native sequence WSX receptor (whether in its native or denatured conformation). Effector functions include ligand binding; and enhancement of survival, differentiation and/or proliferation of cells (especially proliferation of cells). However, effector functions do not include possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against native sequence WSX receptor.

30 "Biological property" when used in conjunction with either "OB protein" or "isolated OB protein" means having an effector function that is directly or indirectly caused or performed by native sequence OB protein. Effector functions of native sequence OB protein include WSX receptor binding and activation; and enhancement of differentiation and/or proliferation of cells expressing this receptor (as determined in the thymidine incorporation assay, for example). A "biologically active" OB protein is one which possesses a
35 biological property of native sequence OB protein.

 A "functional derivative" of a native sequence OB protein is a compound having a qualitative biological property in common with a native sequence OB protein. "Functional derivatives" include, but are not limited to, fragments of native sequence OB proteins and derivatives of native sequence OB proteins and their fragments, provided that they have a biological activity in common with a corresponding native sequence OB protein. The

term "derivative" encompasses both amino acid sequence variants of OB protein and covalent modifications thereof.

The phrase "long half-life" as used in connection with OB derivatives, concerns OB derivatives having a longer plasma half-life and/or slower clearance than a corresponding native sequence OB protein. The long half-life derivatives preferably will have a half-life at least about 1.5-times longer than a native OB protein; more preferably at least about 2-times longer than a native OB protein, more preferably at least about 3-time longer than a native OB protein. The native OB protein preferably is that of the individual to be treated.

An "antigenic function" means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against native sequence WSX receptor. The principal antigenic function of a WSX receptor is that it binds with an affinity of at least about 10^6 L/mole to an antibody raised against native sequence WSX receptor. Ordinarily, the polypeptide binds with an affinity of at least about 10^7 L/mole. The antibodies used to define "antigenic function" are rabbit polyclonal antibodies raised by formulating the WSX receptor in Freund's complete adjuvant, subcutaneously injecting the formulation, and boosting the immune response by intraperitoneal injection of the formulation until the titer of the anti-WSX receptor or antibody plateaus.

"Biologically active" when used in conjunction with either "WSX receptor" or "isolated WSX receptor" means a WSX receptor polypeptide that exhibits or shares an effector function of native sequence WSX receptor and that may (but need not) in addition possess an antigenic function. A principal effector function of the WSX receptor is its ability to induce proliferation of CD34+ human umbilical cord blood cells in the colony assay described in Example 8.

"Antigenically active" WSX receptor is defined as a polypeptide that possesses an antigenic function of WSX receptor and that may (but need not) in addition possess an effector function.

"Percent amino acid sequence identity" is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the native sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the candidate sequence shall be construed as affecting sequence identity or homology.

A "thymidine incorporation assay" can be used to screen for molecules which activate the WSX receptor. In order to perform this assay, IL-3 dependent Baf3 cells (Palacios *et al.*, *Cell*, 41:727-734 (1985)) are stably transfected with full length native sequence WSX receptor as described in Example 4. The WSX receptor/Baf3 cells so generated are starved of IL-3 for, e.g., 24 hours in a humidified incubator at 37°C in 5%CO₂ and air. Following IL-3 starvation, the cells are plated out in 96 well culture dishes with, or without, a test sample containing a potential agonist (such test samples are optionally diluted) and cultured for 24 hours in a cell culture incubator. 20μl of serum free RPMI media containing 1μCi of ³H thymidine is added to each well for the last 6-8 hours. The cells are then harvested in 96 well filter plates and washed with water. The filters are then counted using a Packard Top Count Microplate Scintillation Counter, for example. Agonists are expected to induce a statistically significant increase (to a P value of 0.05) in ³H uptake, relative to control. Preferred agonists leads to an increase in ³H uptake which is at least two fold f that of the control.

An "isolated" WSX receptor nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the WSX receptor nucleic acid. An isolated WSX receptor nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated WSX receptor nucleic acid molecules therefore are distinguished from the WSX receptor nucleic acid molecule as it exists in natural cells. However, an isolated WSX receptor nucleic acid molecule includes WSX receptor nucleic acid molecules contained in cells that ordinarily express WSX receptor where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The expression "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies, antibody compositions with polyepitopic specificity, bispecific antibodies, diabodies, and single-chain molecules, as well as antibody fragments (e.g., Fab, F(ab')₂, and Fv), so long as they exhibit the desired biological activity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the

character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567 (Cabilly *et al.*)). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly *et al.*, *supra*; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Reichmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). The humanized antibody includes a PrimatizedTM antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the

heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

"Non-immunogenic in a human" means that upon contacting the polypeptide of interest in a physiologically acceptable carrier and in a therapeutically effective amount with the appropriate tissue of a human, no state of sensitivity or resistance to the polypeptide of interest is demonstrable upon the second administration of the polypeptide of interest after an appropriate latent period (e.g., 8 to 14 days).

By "agonist antibody" is meant an antibody which is able to activate native sequence WSX receptor. The agonist antibody of particular interest herein is one which mimics one or more (e.g. all) of the biological properties of naturally occurring WSX ligand, OB protein. In preferred embodiments, the agonist antibody has a quantitative biological property of OB protein which is within about two orders of magnitude, and preferably within about one order of magnitude, that of OB protein. The agonist antibody may bind to and activate WSX receptor and thereby stimulate proliferation and/or differentiation and/or maturation and/or survival of a cell which expresses the WSX receptor (e.g. WSX receptor variant 13.2). In this embodiment of the invention, the agonist antibody may be one which enhances proliferation and/or differentiation of a hematopoietic progenitor cell which expresses the WSX receptor at its cell surface; enhances proliferation and/or differentiation of lymphoid blood cell lineages; enhances proliferation and/or differentiation of myeloid blood cell lineages; and/or enhances proliferation and/or differentiation of erythroid blood cell lineages. The agonist antibody may display agonist activity upon binding to a chimeric receptor comprising the WSX receptor extracellular domain in the KIRA ELISA. The agonist antibody may stimulate ^3H uptake in the thymidine incorporation assay using a signaling WSX receptor (see above); decrease body weight and/or fat-depot weight and/or food intake in an obese mammal (e.g. in the *ob/ob* mouse); effect Ca^{2+} influx in adipocytes; and/or activate downstream signaling molecules of OB protein.

A "neutralizing antibody" is one which is able to block or significantly reduce an effector function of native sequence WSX receptor or OB protein. For example, a neutralizing antibody may inhibit or reduce WSX receptor activation by a WSX ligand as determined in the thymidine incorporation assay or in a KIRA ELISA.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., ^{131}I , ^{125}I , ^{90}Y and ^{186}Re), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include Adriamycin, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside ("Ara-C"), Cyclophosphamide, Thiotepa, Taxotere (docetaxel), Busulfan, Cytosine, Taxol, Methotrexate, Cisplatin, Melphalan, Vinblastine, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitoxantrone, Vincristine, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Esperamicins (see U.S. Pat. No. 4,675,187), Melphalan and other related nitrogen mustards.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast (1986)

and Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt *et al.*, (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β -lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

10 An "antagonist" of the WSX receptor and/or OB protein is a molecule which prevents, or interferes with, binding and/or activation of the WSX receptor or OB protein. Such molecules can be screened for their ability to competitively inhibit WSX receptor activation by OB protein in the thymidine incorporation assay disclosed herein, for example. Examples of such molecules include: WSX receptor ECD; WSX receptor immunoadhesin; neutralizing antibodies against WSX receptor or OB protein; small molecule and peptide
15 antagonists; and antisense nucleotides against the WSX receptor or *ob* gene.

The phrase "enhancing proliferation of a cell" encompasses the step of increasing the extent of growth and/or reproduction of the cell relative to an untreated cell either *in vitro* or *in vivo*. An increase in cell proliferation in cell culture can be detected by counting the number of cells before and after exposure to a molecule of interest. The extent of proliferation can be quantified via microscopic examination of the degree
20 of confluency. Cell proliferation can also be quantified using the thymidine incorporation assay described herein.

By "enhancing differentiation of a cell" is meant the act of increasing the extent of the acquisition or possession of one or more characteristics or functions which differ from that of the original cell (*i.e.* cell specialization). This can be detected by screening for a change in the phenotype of the cell (*e.g.*, identifying morphological changes in the cell).

25 A "hematopoietic progenitor cell" or "primitive hematopoietic cell" is one which is able to differentiate to form a more committed or mature blood cell type.

"Lymphoid blood cell lineages" are those hematopoietic precursor cells which are able to differentiate to form lymphocytes (B-cells or T-cells). Likewise, "lymphopoiesis" is the formation of lymphocytes.

"Erythroid blood cell lineages" are those hematopoietic precursor cells which are able to differentiate
30 to form erythrocytes (red blood cells) and "erythropoiesis" is the formation of erythrocytes.

The phrase "myeloid blood cell lineages", for the purposes herein, encompasses all hematopoietic precursor cells, other than lymphoid and erythroid blood cell lineages as defined above, and "myelopoiesis" involves the formation of blood cells (other than lymphocytes and erythrocytes).

A "CD34+ cell population" is enriched for hematopoietic stem cells. A CD34+ cell population can be
35 obtained from umbilical cord blood or bone marrow, for example. Human umbilical cord blood CD34+ cells can be selected for using immunomagnetic beads sold by Miltenyi (California), following the manufacturer's directions.

"Physiologically acceptable" carriers, excipients, or stabilizers are ones which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically

acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, and IgG4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule. Exemplary salvage receptor binding epitope sequences include HQNLSDGK (SEQ ID NO:39); HQNISDGK (SEQ ID NO:40); HQSLGTQ (SEQ ID NO:41); VISSLHGQ (SEQ ID NO:42); and PKNSSMISNTP (SEQ ID NO:43).

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are OB protein; growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- β ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , - β , and - γ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; and other polypeptide factors including leukemia inhibitory factor (LIF) and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

A "lineage-specific cytokine" is one which acts on relatively committed cells in the hematopoietic cascade and gives rise to an expansion in blood cells of a single lineage. Examples of such cytokines include EPO, TPO, and G-CSF.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

The term "obesity" is used to designate a condition of being overweight associated with excessive bodily fat. The desirable weight for a certain individual depends on a number of factors including sex, height, age, overall built, etc. The same factors will determine when an individual is considered obese. The determination of an optimum body weight for a given individual is well within the skill of an ordinary physician.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

By "solid phase" is meant a non-aqueous matrix to which a reagent of interest (*e.g.*, the WSX receptor or an antibody thereto) can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.*, controlled pore glass), polysaccharides (*e.g.*, agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.*, an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

II. Modes for Carrying Out the Invention

The present invention is based on the discovery of the WSX receptor. The experiments described herein demonstrate that this molecule is a cytokine receptor which appears to play a role in enhancing proliferation and/or differentiation of hematopoietic cells. In particular, this receptor has been found to be present in enriched human stem cell populations, thus indicating that WSX ligands, such as agonist antibodies, may be used to stimulate proliferation of hematopoietic stem cells/progenitor cells. Other uses for this receptor will be apparent from the following discussion. A description follows as to how WSX receptor or OB proteins may be prepared.

a. Preparation of WSX Receptor or OB Protein

Techniques suitable for the production of WSX receptor or OB protein are well known in the art and include isolating WSX receptor or OB protein from an endogenous source of the polypeptide, peptide synthesis (using a peptide synthesizer) and recombinant techniques (or any combination of these techniques). The preferred technique for production of WSX receptor or OB protein is a recombinant technique to be described below.

Most of the discussion below pertains to recombinant production of WSX receptor or OB protein by culturing cells transformed with a vector containing WSX receptor or OB protein nucleic acid and recovering the polypeptide from the cell culture. It is further envisioned that the WSX receptor or OB protein of this invention may be produced by homologous recombination, as provided for in WO 91/06667, published 16 May 1991.

Briefly, this method involves transforming primary human cells containing a WSX receptor or OB protein-encoding gene with a construct (*i.e.*, vector) comprising an amplifiable gene (such as dihydrofolate reductase (DHFR) or others discussed below) and at least one flanking region of a length of at least about 150 bp that is homologous with a DNA sequence at the locus of the coding region of the WSX receptor or OB protein gene to provide amplification of the WSX receptor or OB protein gene. The amplifiable gene must be at a site that does not interfere with expression of the WSX receptor or OB protein gene. The transformation is conducted such that the construct becomes homologously integrated into the genome of the primary cells to define an amplifiable region.

Primary cells comprising the construct are then selected for by means of the amplifiable gene or other marker present in the construct. The presence of the marker gene establishes the presence and integration of the

construct into the host genome. No further selection of the primary cells need be made, since selection will be made in the second host. If desired, the occurrence of the homologous recombination event can be determined by employing PCR and either sequencing the resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when DNA from correct homologous integrants is present and expanding only those cells containing such fragments. Also if desired, the selected cells may be amplified at this point by stressing the cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the amplification step is not conducted until after the second transformation described below.

After the selection step, DNA portions of the genome, sufficiently large to include the entire amplifiable region, are isolated from the selected primary cells. Secondary mammalian expression host cells are then transformed with these genomic DNA portions and cloned, and clones are selected that contain the amplifiable region. The amplifiable region is then amplified by means of an amplifying agent if not already amplified in the primary cells. Finally, the secondary expression host cells now comprising multiple copies of the amplifiable region containing WSX receptor or OB protein are grown so as to express the gene and produce the protein.

i. **Isolation of DNA Encoding WSX Receptor or OB Protein**

The DNA encoding WSX receptor or OB protein may be obtained from any cDNA library prepared from tissue believed to possess the WSX receptor or OB protein mRNA and to express it at a detectable level. Accordingly, WSX receptor or OB protein DNA can be conveniently obtained from a cDNA library prepared from mammalian fetal liver. The WSX receptor or OB protein-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries are screened with probes (such as antibodies to the WSX receptor or OB protein, or oligonucleotides of about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding WSX receptor or OB protein is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various human tissues, preferably human fetal liver. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide must be labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ³²P-labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Amino acid sequence variants of WSX receptor or OB protein are prepared by introducing appropriate nucleotide changes into the WSX receptor or OB protein DNA, or by synthesis of the desired WSX receptor or OB protein. Such variants represent insertions, substitutions, and/or specified deletions of, residues within or at one or both of the ends of the amino acid sequence of a naturally occurring human WSX receptor or OB protein, such as the WSX receptor variants shown in Figs 2A-B or the human OB protein of Zhang *et al.*, *supra*.

Preferably, these variants represent insertions and/or substitutions within r at one or both ends of the mature sequence, and/or insertions, substitutions and/or specified deletions within or at one or both of the ends of the signal sequence of the WSX receptor or OB protein. Any combination of insertion, substitution, and/or specified deletion is made to arrive at the final c nstruct, provided that the final construct p sses the desired biological activity as defined herein. The amino acid changes also may alter post-translational processes of the WSX receptor or OB protein, such as changing the number or position of glycosylation sites, altering the membrane anchoring characteristics, and/or altering the intracellular location of the WSX receptor or OB protein by inserting, deleting, or otherwise affecting the leader sequence of the WSX receptor or OB protein.

Variations in the native sequence as described above can be made using any of the techniques and guidelines for conservative and non-conservative mutations set forth in U.S. Pat. No. 5,364,934. These include oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. See also, for example, Table I therein and the discussion surrounding this table for guidance on selecting amino acids to change, add, or delete.

ii. Insertion of Nucleic Acid into Replicable Vector

The nucleic acid (e.g., cDNA or genomic DNA) encoding the WSX receptor or OB protein is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(1) Signal sequence component

The WSX receptor or OB proteins of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the WSX receptor or OB protein DNA that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native WSX receptor or OB protein signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, e.g., the yeast invertase leader, α factor leader (including *Saccharomyces* and *Kluyveromyces* α -factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence (e.g., the WSX receptor or OB protein presequence that normally directs secretion of WSX receptor or OB protein from human cells *in vivo*) is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from other animal WSX receptors or OB proteins, and signal sequences from secreted polypeptides of the same r related species, as well as viral secretory leaders, for example, the herpes simplex gD signal.

The DNA for such precursor region is ligated in reading frame to DNA encoding the mature WSX receptor r OB protein.

(2) *Origin of replication component*

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of WSX receptor or OB protein DNA. However, the recovery of genomic DNA encoding WSX receptor or OB protein is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the WSX receptor or OB protein DNA.

(3) *Selection gene component*

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the WSX receptor or OB protein nucleic acid, such as DHFR or thymidine kinase. The mammalian cell transformants are placed under selection pressure that only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes WSX receptor or OB protein. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of WSX receptor or OB protein are synthesized from the

amplified DNA. Other examples of amplifiable genes include metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, *etc.*

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980). The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding WSX receptor or OB protein. This amplification technique can be used with any otherwise suitable host, *e.g.*, ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding WSX receptor or OB protein, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, *Nature* 282:39 (1979)). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics* 85:12 (1977). The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

In addition, vectors derived from the 1.6 μ m circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Bianchi *et al.*, *Curr. Genet.* 12:185 (1987). More recently, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, *Bio/Technology* 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer *et al.*, *Bio/Technology* 9:968-975 (1991).

(4) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the WSX receptor or OB protein nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence, such as the WSX receptor or OB protein nucleic acid sequence, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, *e.g.*, the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to WSX receptor or OB protein-encoding DNA by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter

sequence into the vector. Both the native WSX receptor or OB protein promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the WSX receptor or OB protein DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of WSX receptor or OB protein as compared to the native WSX receptor or OB protein promoter.

5 Promoters suitable for use with prokaryotic hosts include the β -lactamase and lactose promoter systems (Chang *et al.*, *Nature* 275:615 (1978); Goeddel *et al.*, *Nature* 281:544 (1979)), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.* 8:4057 (1980); EP 36,776), and hybrid promoters such as the tac promoter. deBoer *et al.*, *Proc. Natl. Acad. Sci. USA* 80:21-25 (1983). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled
10 worker operably to ligate them to DNA encoding WSX receptor or OB protein (Siebenlist *et al.*, *Cell* 20:269 (1980)) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding WSX receptor or OB protein.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region
15 located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

20 Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, *J. Biol. Chem.* 255:2073 (1980)) or other glycolytic enzymes (Hess *et al.*, *J. Adv. Enzyme Reg.* 7:149 (1968); Holland, *Biochemistry* 17:4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose
25 isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and
30 promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

WSX receptor or OB protein transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma
35 virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the WSX receptor or OB protein sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, *Nature* 273:113 (1978); Mulligan *et al.*, *Science* 209:1422-1427 (1980); Pavlakis *et al.*, *Proc. Natl. Acad. Sci. USA* 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway *et al.*, *Gene* 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described in U.S. Patent No. 4,601,978. See also Gray *et al.*, *Nature* 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; Reyes *et al.*, *Nature* 297:598-601 (1982) on expression of human β -interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus; Canaani *et al.*, *Proc. Natl. Acad. Sci. USA* 79:5166-5170 (1982) on expression of the human interferon β 1 gene in cultured mouse and rabbit cells; and Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

15 (5) *Enhancer element component*

Transcription of a DNA encoding the WSX receptor or OB protein of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent, having been found 5' (Laimins *et al.*, *Proc. Natl. Acad. Sci. USA* 78:993 (1981)) and 3' (Lusky *et al.*, *Mol. Cell Bio.* 3:1108 (1983)) to the transcription unit, within an intron (Banerji *et al.*, *Cell* 33:729 (1983)), as well as within the coding sequence itself. Osborne *et al.*, *Mol. Cell Bio.* 4:1293 (1984). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the WSX receptor or OB protein-encoding sequence, but is preferably located at a site 5' from the promoter.

30 (6) *Transcription termination component*

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding WSX receptor or OB protein.

35 (7) *Construction and analysis of vectors*

Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing *et al.*, *Nucleic Acids Res.* 9:309 (1981) or
 5 by the method of Maxam *et al.*, *Methods in Enzymology* 65:499 (1980).

Transient expression vectors

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding WSX receptor or OB protein. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell
 10 accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Sambrook *et al.*, *supra*, pp. 16.17 - 16.22. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention
 15 for purposes of identifying analogs and variants of WSX receptor or OB protein that are biologically active WSX receptor or OB protein.

(8) *Suitable exemplary vertebrate cell vectors*

Other methods, vectors, and host cells suitable for adaptation to the synthesis of WSX receptor or OB protein in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature* 293:620-625 (1981); Mantei
 20 *et al.*, *Nature* 281:40-46 (1979); EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of WSX receptor or OB protein is pRK5 (EP 307,247) or pSVI6B. WO 91/08291 published 13 June 1991.

iii. *Selection and Transformation of Host Cells*

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast,
 25 or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One
 30 preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Strain W3110 is a particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell should secrete minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding
 35 proteins, with examples of such hosts including *E. coli* W3110 strain 27C7. The complete genotype of 27C7 is *tonA Δ ptr3 phoA Δ E15 Δ (argF-lac)169 ompT Δ degP41 kar^r*. Strain 27C7 was deposited on 30 October 1991 in the American Type Culture Collection as ATCC No. 55,244. Alternatively, the strain of *E. coli* having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990 may be employed. Alternatively still, methods of cloning, *e.g.*, PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for WSX receptor or OB protein-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* (Beach *et al.*, *Nature*, 290:140 (1981); EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, *supra*) such as, *e.g.*, *K. lactis* (MW98-8C, CBS683, CBS4574), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906; Van den Berg *et al.*, *supra*), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al.*, *J. Basic Microbiol.* 28:265-278 (1988)); *Candida*; *Trichoderma reesei* (EP 244,234); *Neurospora crassa* (Case *et al.*, *Proc. Natl. Acad. Sci. USA* 76:5259-5263 (1979)); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, *e.g.*, *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, *Biochem. Biophys. Res. Commun.* 112:284-289 (1983); Tilburn *et al.*, *Gene* 26:205-221 (1983); Yelton *et al.*, *Proc. Natl. Acad. Sci. USA* 81:1470-1474 (1984)) and *A. niger*. Kelly *et al.*, *EMBO J.* 4:475-479 (1985).

Suitable host cells for the expression of glycosylated WSX receptor or OB protein are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. See, *e.g.*, Luckow *et al.*, *Bio/Technology* 6:47-55 (1988); Miller *et al.*, in *Genetic Engineering*, Setlow *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, *Nature* 315:592-594 (1985). A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the WSX receptor or OB protein-encoding DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding the WSX receptor or OB protein is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the WSX receptor or OB protein-encoding DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, *J. Mol. Appl. Gen.* 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. See, *e.g.*, *Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973). Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40

(COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (WI38, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

10 Host cells are transfected and preferably transformed with the above-described expression or cloning vectors for WSX receptor or OB protein production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, CaPO_4 and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene* 23:315 (1983) and WO 89/05859 published 29 June 1989. In addition, plants may be transfected using ultrasound treatment as described in WO 91/00358 published 10 January 1991.

For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham *et al.*, *Virology* 52:456-457 (1978) is preferred. General aspects of mammalian cell host system transformations have been described in U.S. Pat. No. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.* 130:946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. USA* 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, *etc.*, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology* 185:527-537 (1990) and Mansour *et al.*, *Nature* 336:348-352 (1988).

iv. Culturing the Host Cells

35 Prokaryotic cells used to produce the WSX receptor or OB protein of this invention are cultured in suitable media as described generally in Sambrook *et al.*, *supra*.

The mammalian host cells used to produce the WSX receptor or OB protein of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma)

are suitable for culturing the host cells. In addition, any of the media described in Ham *et al. Meth. Enz.* 58:44 (1979), Barnes *et al., Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as GENTAMYCINTM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art.

The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

In general, principles, protocols, and practical techniques for maximizing the productivity of mammalian cell cultures can be found in *Mammalian Cell Biotechnology: a Practical Approach*, M. Butler, ed. (IRL Press, 1991).

The host cells referred to in this disclosure encompass cells in culture as well as cells that are within a host animal.

v. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA* 77:5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ³²P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al., Am. J. Clin. Path.* 75:734-738 (1980).

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared as described herein.

vi. **Purification of WSX Receptor or OB Protein**

WSX receptor (*e.g.*, WSX receptor ECD) or OB protein preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates. If the WSX receptor is membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton-X 100)

5 When WSX receptor or OB protein is produced in a recombinant cell other than one of human origin, the WSX receptor or OB protein is completely free of proteins or polypeptides of human origin. However, it is necessary to purify WSX receptor or OB protein from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to WSX receptor or OB protein. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. WSX receptor or OB protein thereafter is
10 purified from contaminant soluble proteins and polypeptides, with the following procedures being exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75™; and protein A Sepharose™ columns to remove contaminants such as IgG.

15 WSX receptor or OB protein variants in which residues have been deleted, inserted, or substituted are recovered in the same fashion as native sequence WSX receptor or OB protein, taking account of any substantial changes in properties occasioned by the variation. Immunoaffinity columns such as a rabbit polyclonal anti-WSX receptor or OB protein column can be employed to absorb the WSX receptor or OB protein variant by binding it to at least one remaining immune epitope.

20 A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants.

vii. **Covalent Modifications**

Covalent modifications of WSX receptor or OB protein are included within the scope of this invention.

25 Both native sequence WSX receptor or OB protein and amino acid sequence variants of the WSX receptor or OB protein may be covalently modified. One type of covalent modification of the WSX receptor or OB protein is introduced into the molecule by reacting targeted amino acid residues of the WSX receptor or OB protein with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the WSX receptor or OB protein.

30 Cysteinyll residues most commonly are reacted with α -haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyll residues also are derivatized by reaction with bromotrifluoroacetone, α -bromo- β -(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

35 Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyll and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyll residues. Other suitable

reagents for derivatizing α -amino-containing residues include imidoesters such as methyl picolinimate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4-pentanedione, and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed under alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as with the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidazole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides ($R-N=C=N-R'$), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking WSX receptor or OB protein to a water-insoluble support matrix or surface for use in the method for purifying anti-WSX receptor or OB protein antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidyl)propionate, and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-((p-azidophenyl)dithio)propioimide yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Patent Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. These residues are deamidated under neutral or basic conditions. The deamidated form of these residues falls within the scope of this invention.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the WSX receptor or OB protein included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in native WSX receptor or OB protein, and/or adding one or more glycosylation sites that are not present in the native WSX receptor or OB protein.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxylamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the WSX receptor or OB protein is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the native WSX receptor or OB protein sequence (for O-linked glycosylation sites). For ease, the WSX receptor or OB protein amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the WSX receptor or OB protein at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above and in U.S. Pat. No. 5,364,934, *supra*.

Another means of increasing the number of carbohydrate moieties on the WSX receptor or OB protein is by chemical or enzymatic coupling of glycosides to the polypeptide. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published 11 September 1987, and in Aplin *et al.*, *CRC Crit. Rev. Biochem.* 259-306 (1981).

Removal of carbohydrate moieties present on the WSX receptor or OB protein may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin, *et al.*, *Arch. Biochem. Biophys.* 259:52 (1987) and by Edge *et al.*, *Anal. Biochem.* 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.* 138:350 (1987).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.*, *J. Biol. Chem.* 257:3105 (1982). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of WSX receptor or OB protein comprises linking the WSX receptor or OB protein to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

Since it is often difficult to predict in advance the characteristics of a variant WSX receptor or OB protein, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. A change in the immunological character of the WSX receptor or OB protein molecule, such as affinity for a given antibody, is also able to be measured by a competitive-type immunoassay. The WSX receptor variant is assayed for changes in the ability of the protein to induce cell proliferation in the colony assay of Example 8. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

viii. Epitope-Tagged WSX Receptor or OB Protein

This invention encompasses chimeric polypeptides comprising WSX receptor or OB protein fused to a heterologous polypeptide. A chimeric WSX receptor or OB protein is one type of WSX receptor or OB protein variant as defined herein. In one preferred embodiment, the chimeric polypeptide comprises a fusion of the WSX receptor or OB protein with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally provided at the amino- or carboxyl- terminus of the WSX receptor or OB protein. Such epitope-tagged forms of the WSX receptor or OB protein are desirable as the presence thereof can be detected using a labeled antibody against the tag polypeptide. Also, provision of the epitope tag enables the WSX receptor or OB protein to be readily purified by affinity purification using the anti-tag antibody. Affinity purification techniques and diagnostic assays involving antibodies are described later herein.

Tag polypeptides and their respective antibodies are well known in the art. Examples include the flu HA tag polypeptide and its antibody 12CA5 (Field *et al.*, *Mol. Cell. Biol.* 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al.*, *Molecular and Cellular Biology* 5:3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody. Paborsky *et al.*, *Protein Engineering* 3(6):547-553 (1990). Other tag polypeptides have been disclosed. Examples include the Flag-peptide (Hopp *et al.*, *BioTechnology* 6:1204-1210 (1988)); the KT3 epitope peptide (Martin *et al.*, *Science* 255:192-194 (1992)); an α -tubulin epitope peptide (Skinner *et al.*, *J. Biol. Chem.* 266:15163-15166 (1991)); and the T7 gene 10 protein peptide tag. Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA* 87:6393-6397 (1990). Once the tag polypeptide has been selected, an antibody thereto can be generated using the techniques disclosed herein.

The general methods suitable for the construction and production of epitope-tagged WSX receptor or OB protein are the same as those disclosed hereinabove. WSX receptor or OB protein-tag polypeptide fusions are most conveniently constructed by fusing the cDNA sequence encoding the WSX receptor or OB protein portion in-frame to the tag polypeptide DNA sequence and expressing the resultant DNA fusion construct in appropriate host cells. Ordinarily, when preparing the WSX receptor or OB protein-tag polypeptide chimeras of the present invention, nucleic acid encoding the WSX receptor or OB protein will be fused at its 3' end to nucleic acid encoding the N-terminus of the tag polypeptide, however 5' fusions are also possible.

Epitope-tagged WSX receptor or OB protein can be conveniently purified by affinity chromatography using the anti-tag antibody. The matrix to which the affinity antibody is attached is most often agarose, but other matrices are available (e.g. controlled pore glass or poly(styrenedivinyl)benzene). The epitope-tagged WSX

receptor or OB protein can be eluted from the affinity column by varying the buffer pH or ionic strength or adding chaotropic agents, for example.

ix. WSX Receptor or OB Protein Immunoadhesins

Chimeras constructed from a receptor sequence linked to an appropriate immunoglobulin constant domain sequence (immunoadhesins) are known in the art. Immunoadhesins reported in the literature include fusions of the T cell receptor* (Gascoigne *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 2936-2940 (1987)); CD4* (Capon *et al.*, *Nature* 337: 525-531 (1989); Traunecker *et al.*, *Nature* 339: 68-70 (1989); Zettmeissl *et al.*, *DNA Cell Biol.* USA 9: 347-353 (1990); Byrn *et al.*, *Nature* 344: 667-670 (1990)); L-selectin (homing receptor) ((Watson *et al.*, *J. Cell. Biol.* 110:2221-2229 (1990); Watson *et al.*, *Nature* 349: 164-167 (1991)); CD44* (Aruffo *et al.*, *Cell* 61: 1303-1313 (1990)); CD28* and B7* (Linsley *et al.*, *J. Exp. Med.* 173: 721-730 (1991)); CTLA-4* (Lisley *et al.*, *J. Exp. Med.* 174: 561-569 (1991)); CD22* (Stamenkovic *et al.*, *Cell* 66:1133-1144 (1991)); TNF receptor (Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 10535-10539 (1991); Lesslauer *et al.*, *Eur. J. Immunol.* 27: 2883-2886 (1991); Peppel *et al.*, *J. Exp. Med.* 174:1483-1489 (1991)); NP receptors (Bennett *et al.*, *J. Biol. Chem.* 266:23060-23067 (1991)); and IgE receptor α * (Ridgway *et al.*, *J. Cell. Biol.* 115:abstr. 1448 (1991)), where the asterisk (*) indicates that the receptor is member of the immunoglobulin superfamily.

The simplest and most straightforward immunoadhesin design combines the binding region(s) of the "adhesin" protein with the hinge and Fc regions of an immunoglobulin heavy chain. Ordinarily, when preparing the WSX receptor or OB-immunoglobulin chimeras of the present invention, nucleic acid encoding OB protein or the extracellular domain of the WSX receptor will be fused C-terminally to nucleic acid encoding the N-terminus of an immunoglobulin constant domain sequence, however N-terminal fusions are also possible. For OB-immunoglobulin chimeras, an OB protein fragment which retains the ability to bind to the WSX receptor may be employed.

Typically, in such fusions the encoded chimeric polypeptide will retain at least functionally active hinge, CH2 and CH3 domains of the constant region of an immunoglobulin heavy chain. Fusions are also made to the C-terminus of the Fc portion of a constant domain, or immediately N-terminal to the CH1 of the heavy chain or the corresponding region of the light chain.

The precise site at which the fusion is made is not critical; particular sites are well known and may be selected in order to optimize the biological activity, secretion or binding characteristics of the WSX receptor or OB-immunoglobulin chimeras.

In some embodiments, the WSX receptor or OB-immunoglobulin chimeras are assembled as monomers, or hetero- or homo-multimers, and particularly as dimers or tetramers, essentially as illustrated in WO 91/08298.

In a preferred embodiment, the OB protein sequence or WSX receptor extracellular domain sequence is fused to the N-terminus of the C-terminal portion of an antibody (in particular the Fc domain), containing the effector functions of an immunoglobulin, e.g. immunoglobulin G1 (IgG1). It is possible to fuse the entire heavy chain constant region to the OB protein or WSX receptor extracellular domain sequence. However, more preferably, a sequence beginning in the hinge region just upstream of the papain cleavage site (which defines IgG Fc chemically; residue 216, taking the first residue of heavy chain constant region to be 114, or analogous sites of other immunoglobulins) is used in the fusion. In a particularly preferred embodiment, the OB protein or WSX

receptor amino acid sequence is fused to the hinge region, CH2 and CH3, or the CH1, hinge, CH2 and CH3 domains of an IgG1, IgG2, or IgG3 heavy chain. The precise site at which the fusion is made is not critical, and the optimal site can be determined by routine experimentation.

In some embodiments, the WSX receptor or OB-immunoglobulin chimeras are assembled as multimers, and particularly as homo-dimers or -tetramers. Generally, these assembled immunoglobulins will have known unit structures. A basic four chain structural unit is the form in which IgG, IgD, and IgE exist. A four unit is repeated in the higher molecular weight immunoglobulins; IgM generally exists as a pentamer of basic four units held together by disulfide bonds. IgA globulin, and occasionally IgG globulin, may also exist in multimeric form in serum. In the case of multimer, each four unit may be the same or different.

Various exemplary assembled WSX receptor or OB-immunoglobulin chimeras within the scope herein are schematically diagrammed below:

- (a) AC_L-AC_L ;
- (b) $AC_H-(AC_H, AC_L-AC_H, AC_L-V_HC_H, \text{ or } V_LC_L-AC_H)$;
- (c) $AC_L-AC_H-(AC_L-AC_H, AC_L-V_HC_H, V_LC_L-AC_H, \text{ or } V_LC_L-V_HC_H)$;
- (d) $AC_L-V_HC_H-(AC_H, \text{ or } AC_L-V_HC_H, \text{ or } V_LC_L-AC_H)$;
- (e) $V_LC_L-AC_H-(AC_L-V_HC_H, \text{ or } V_LC_L-AC_H)$; and
- (f) $(A-Y)_n-(V_LC_L-V_HC_H)_2$,

wherein

each A represents identical or different OB protein or WSX receptor amino acid sequences;

V_L is an immunoglobulin light chain variable domain;

V_H is an immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_H is an immunoglobulin heavy chain constant domain;

n is an integer greater than 1;

Y designates the residue of a covalent cross-linking agent.

In the interests of brevity, the foregoing structures only show key features; they do not indicate joining (J) or other domains of the immunoglobulins, nor are disulfide bonds shown. However, where such domains are required for binding activity, they shall be constructed as being present in the ordinary locations which they occupy in the immunoglobulin molecules.

Alternatively, the OB protein or WSX receptor extracellular domain sequence can be inserted between immunoglobulin heavy chain and light chain sequences such that an immunoglobulin comprising a chimeric heavy chain is obtained. In this embodiment, the OB protein or WSX receptor sequence is fused to the 3' end of an immunoglobulin heavy chain in each arm of an immunoglobulin, either between the hinge and the CH2 domain, or between the CH2 and CH3 domains. Similar constructs have been reported by Hoogenboom *et al.*, *Mol. Immunol.*, 28:1027-1037 (1991).

Although the presence of an immunoglobulin light chain is not required in the immunoadhesins of the present invention, an immunoglobulin light chain might be present either covalently associated to an OB protein or WSX receptor-immunoglobulin heavy chain fusion polypeptide, or directly fused to the WSX receptor extracellular domain or OB protein. In the former case, DNA encoding an immunoglobulin light chain is

typically coexpressed with the DNA encoding the OB protein or WSX receptor-immunoglobulin heavy chain fusion protein. Upon secretion, the hybrid heavy chain and the light chain will be covalently associated to provide an immunoglobulin-like structure comprising two disulfide-linked immunoglobulin heavy chain-light chain pairs. Methods suitable for the preparation of such structures are, for example, disclosed in U.S. Patent
5 No. 4,816,567 issued 28 March 1989.

In a preferred embodiment, the immunoglobulin sequences used in the construction of the immunoadhesins of the present invention are from an IgG immunoglobulin heavy chain constant domain. For human immunoadhesins, the use of human IgG1 and IgG3 immunoglobulin sequences is preferred. A major advantage of using IgG1 is that IgG1 immunoadhesins can be purified efficiently on immobilized protein A. In
10 contrast, purification of IgG3 requires protein G, a significantly less versatile medium. However, other structural and functional properties of immunoglobulins should be considered when choosing the Ig fusion partner for a particular immunoadhesin construction. For example, the IgG3 hinge is longer and more flexible, so it can accommodate larger adhesion domains that may not fold or function properly when fused to IgG1. Another consideration may be valency; IgG immunoadhesins are bivalent homodimers, whereas Ig subtypes like IgA and
15 IgM may give rise to dimeric or pentameric structures, respectively, of the basic Ig homodimer unit. For immunoadhesins designed for *in vivo* application, the pharmacokinetic properties and the effector functions specified by the Fc region are important as well. Although IgG1, IgG2 and IgG4 all have *in vivo* half-lives of 21 days, their relative potencies at activating the complement system are different. IgG4 does not activate complement, and IgG2 is significantly weaker at complement activation than IgG1. Moreover, unlike IgG1,
20 IgG2 does not bind to Fc receptors on mononuclear cells or neutrophils. While IgG3 is optimal for complement activation, its *in vivo* half-life is approximately one third of the other IgG isotypes. Another important consideration for immunoadhesins designed to be used as human therapeutics is the number of allotypic variants of the particular isotype. In general, IgG isotypes with fewer serologically-defined allotypes are preferred. For example, IgG1 has only four serologically-defined allotypic sites, two of which (G1m and 2) are located in the
25 Fc region; and one of these sites G1m1, is non-immunogenic. In contrast, there are 12 serologically-defined allotypes in IgG3, all of which are in the Fc region; only three of these sites (G3m5, 11 and 21) have one allotype which is nonimmunogenic. Thus, the potential immunogenicity of a γ 3 immunoadhesin is greater than that of a γ 1 immunoadhesin.

With respect to the parental immunoglobulin, a useful joining point is just upstream of the cysteines of
30 the hinge that form the disulfide bonds between the two heavy chains. In a frequently used design, the codon for the C-terminal residue of the WSX receptor or OB protein part of the molecule is placed directly upstream of the codons for the sequence DKTHTCPPCP (SEQ ID NO:44) of the IgG1 hinge region.

The general methods suitable for the construction and expression of immunoadhesins are the same as those disclosed hereinabove with regard to WSX receptor and OB protein. Immunoadhesins are most
35 conveniently constructed by fusing the cDNA sequence encoding the WSX receptor or OB protein portion in-frame to an Ig cDNA sequence. However, fusion to genomic Ig fragments can also be used (see, e.g., Gascoigne *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:2936-2940 (1987); Aruff *et al.*, *Cell* 61:1303-1313 (1990); Stamenkovic *et al.*, *Cell* 66:1133-1144 (1991)). The latter type of fusion requires the presence of Ig regulatory sequences for expression. cDNAs encoding IgG heavy-chain constant regions can be isolated based on published sequence

from cDNA libraries derived from spleen or peripheral blood lymphocytes, by hybridization or by polymerase chain reaction (PCR) techniques. The cDNAs encoding the WSX receptor or OB protein and Ig parts of the immunoadhesin are inserted in tandem into a plasmid vector that directs efficient expression in the chosen host cells. For expression in mammalian cells, pRK5-based vectors (Schall *et al.*, *Cell* 61:361-370 (1990)) and
5 CDM8-based vectors (Seed, *Nature* 329:840 (1989)) can be used. The exact junction can be created by removing the extra sequences between the designed junction codons using oligonucleotide-directed deletional mutagenesis (Zoller *et al.*, *Nucleic Acids Res.* 10:6487 (1982); Capon *et al.*, *Nature* 337:525-531 (1989)). Synthetic oligonucleotides can be used, in which each half is complementary to the sequence on either side of the desired junction; ideally, these are 36 to 48-mers. Alternatively, PCR techniques can be used to join the two
10 parts of the molecule in-frame with an appropriate vector.

The choice of host cell line for the expression of the immunoadhesin depends mainly on the expression vector. Another consideration is the amount of protein that is required. Milligram quantities often can be produced by transient transfections. For example, the adenovirus EIA-transformed 293 human embryonic kidney cell line can be transfected transiently with pRK5-based vectors by a modification of the calcium phosphate
15 method to allow efficient immunoadhesin expression. CDM8-based vectors can be used to transfect COS cells by the DEAE-dextran method (Aruffo *et al.*, *Cell* 61:1303-1313 (1990); Zettmeissl *et al.*, *DNA Cell Biol.* US 9:347-353 (1990)). If larger amounts of protein are desired, the immunoadhesin can be expressed after stable transfection of a host cell line. For example, a pRK5-based vector can be introduced into Chinese hamster ovary (CHO) cells in the presence of an additional plasmid encoding dihydrofolate reductase (DHFR) and conferring
20 resistance to G418. Clones resistant to G418 can be selected in culture; these clones are grown in the presence of increasing levels of DHFR inhibitor methotrexate; clones are selected, in which the number of gene copies encoding the DHFR and immunoadhesin sequences is co-amplified. If the immunoadhesin contains a hydrophobic leader sequence at its N-terminus, it is likely to be processed and secreted by the transfected cells. The expression of immunoadhesins with more complex structures may require uniquely suited host cells; for
25 example, components such as light chain or J chain may be provided by certain myeloma or hybridoma cell hosts (Gascoigne *et al.*, 1987, *supra*, Martin *et al.*, *J. Virol.* 67:3561-3568 (1993)).

Immunoadhesins can be conveniently purified by affinity chromatography. The suitability of protein A as an affinity ligand depends on the species and isotype of the immunoglobulin Fc domain that is used in the chimera. Protein A can be used to purify immunoadhesins that are based on human $\gamma 1$, $\gamma 2$, or $\gamma 4$ heavy chains
30 (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human $\gamma 3$ (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. The conditions for binding an immunoadhesin to the protein A or G affinity column are dictated
35 entirely by the characteristics of the Fc domain; that is, its species and isotype. Generally, when the proper ligand is chosen, efficient binding occurs directly from unconditioned culture fluid. One distinguishing feature of immunoadhesins is that, for human $\gamma 1$ molecules, the binding capacity for protein A is somewhat diminished relative to an antibody of the same Fc type. Bound immunoadhesin can be efficiently eluted either at acidic pH

(at or above 3.0), or in a neutral pH buffer containing a mildly chaotropic salt. This affinity chromatography step can result in an immunoadhesin preparation that is >95% pure.

Other methods known in the art can be used in place of, or in addition to, affinity chromatography on protein A or G to purify immunoadhesins. Immunoadhesins behave similarly to antibodies in thiophilic gel chromatography (Hutchens *et al.*, *Anal. Biochem.* 159:217-226 (1986)) and immobilized metal chelate chromatography (Al-Mashikhi *et al.*, *J. Dairy Sci.* 71:1756-1763 (1988)). In contrast to antibodies, however, their behavior on ion exchange columns is dictated not only by their isoelectric points, but also by a charge dipole that may exist in the molecules due to their chimeric nature.

If desired, the immunoadhesins can be made bispecific. Thus, the immunoadhesins of the present invention may combine a WSX receptor extracellular domain and a domain, such as the extracellular domain, of another cytokine receptor subunit. Exemplary cytokine receptors from which such bispecific immunoadhesin molecules can be made include TPO (or *mpl* ligand), EPO, G-CSF, IL-4, IL-7, GH, PRL, IL-3, GM-CSF, IL-5, IL-6, LIF, OSM, CNTF and IL-2 receptors. Alternatively, an OB protein domain may be combined with another cytokine, such as those exemplified herein, in the generation of a bispecific immunoadhesin. For bispecific molecules, trimeric molecules, composed of a chimeric antibody heavy chain in one arm and a chimeric antibody heavy chain-light chain pair in the other arm of their antibody-like structure are advantageous, due to ease of purification. In contrast to antibody-producing quadromas traditionally used for the production of bispecific immunoadhesins, which produce a mixture of ten tetramers, cells transfected with nucleic acid encoding the three chains of a trimeric immunoadhesin structure produce a mixture of only three molecules, and purification of the desired product from this mixture is correspondingly easier.

x. Long Half-Life Derivatives of OB Protein

Preferred OB protein functional derivatives for use in the methods of the present invention include OB-immunoglobulin chimeras (immunoadhesins) and other longer half-life molecules. Techniques for generating OB protein immunoadhesins have been described above. The preferred OB immunoadhesin is made according to the techniques described in Example 11 below.

Other derivatives of the OB proteins, which possess a longer half-life than the native molecules comprise the OB protein or an OB-immunoglobulin chimera covalently bonded to a nonproteinaceous polymer. The nonproteinaceous polymer ordinarily is a hydrophilic synthetic polymer, *i.e.*, a polymer not otherwise found in nature. However, polymers which exist in nature and are produced by recombinant or *in vitro* methods are useful, as are polymers which are isolated from native sources. Hydrophilic polyvinyl polymers fall within the scope of this invention, *e.g.* polyvinylalcohol and polyvinylpyrrolidone. Particularly useful are polyalkylene ethers such as polyethylene glycol (PEG); polyalkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene (Pluronics™); polymethacrylates; carbomers; branched or unbranched polysaccharides which comprise the saccharide monomers D-mannose, D- and L-galactose, fucose, fructose, D-xylose, L-arabinose, D-glucuronic acid, sialic acid, D-galacturonic acid, D-mannuronic acid (*e.g.* polymannuronic acid, or alginic acid), D-glucosamine, D-galactosamine, D-glucose and neuraminic acid including homopolysaccharides and heteropolysaccharides such as lactose, amylopectin, starch, hydroxyethyl starch, amylose, dextran sulfate, dextran, dextrans, glycogen, or the polysaccharide subunit of acid mucopolysaccharides, *e.g.* hyaluronic acid; polymers of sugar alcohols such as poly sorbitol and polymannitol;

heparin or heparon. The polymer prior to cross-linking need not be, but preferably is, water soluble, but the final conjugate must be water soluble. In addition, the polymer should not be highly immunogenic in the conjugate form, nor should it possess viscosity that is incompatible with intravenous infusion or injection if it is intended to be administered by such routes.

5 Preferably the polymer contains only a single group which is reactive. This helps to avoid cross-linking of protein molecules. However, it is within the scope herein to optimize reaction conditions to reduce cross-linking, or to purify the reaction products through gel filtration or chromatographic sieves to recover substantially homogenous derivatives.

10 The molecular weight of the polymer may desirably range from about 100 to 500,000, and preferably is from about 1,000 to 20,000. The molecular weight chosen will depend upon the nature of the polymer and the degree of substitution. In general, the greater the hydrophilicity of the polymer and the greater the degree of substitution, the lower the molecular weight that can be employed. Optimal molecular weights will be determined by routine experimentation.

15 The polymer generally is covalently linked to the OB protein or to the OB-immunoglobulin chimera though a multifunctional crosslinking agent which reacts with the polymer and one or more amino acid or sugar residues of the OB protein or OB-immunoglobulin chimera to be linked. However, it is within the scope of the invention to directly crosslink the polymer by reacting a derivatized polymer with the hybrid, or via versa.

20 The covalent crosslinking site on the OB protein or OB-immunoglobulin chimera includes the N-terminal amino group and epsilon amino groups found on lysine residues, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. The polymer may be covalently bonded directly to the hybrid without the use of a multifunctional (ordinarily bifunctional) crosslinking agent. Covalent binding to amino groups is accomplished by known chemistries based upon cyanuric chloride, carbonyl diimidazole, aldehyde reactive groups (PEG alkoxide plus diethyl acetal of bromoacetaldehyde; PEG plus DMSO and acetic anhydride, or PEG chloride plus the phenoxide of 4-hydroxybenzaldehyde, succinimidyl active esters, activated dithiocarbonate PEG, 2,4,5-trichlorophenylchloroformate or P-nitrophenylchloroformate activated PEG). Carboxyl groups are derivatized by coupling PEG-amine using carbodiimide.

25 Polymers are conjugated to oligosaccharide groups by oxidation using chemicals, e.g. metaperiodate, or enzymes, e.g. glucose or galactose oxidase (either of which produces the aldehyde derivative of the carbohydrate), followed by reaction with hydrazide or amino derivatized polymers, in the same fashion as is described by Heitzmann *et al.*, *P.N.A.S.* 71:3537-41 (1974) or Bayer *et al.*, *Methods in Enzymology* 62:310 (1979), for the labeling of oligosaccharides with biotin or avidin. Further, other chemical or enzymatic methods which have been used heretofore to link oligosaccharides are particularly advantageous because, in general, there are fewer substitutions than amino acid sites for derivatization, and the oligosaccharide products thus will be more homogenous. The oligosaccharide substituents also are optionally modified by enzyme digestion to remove sugars, e.g. by neuraminidase digestion, prior to polymer derivatization.

35 The polymer will bear a group which is directly reactive with an amino acid side chain, or the N- or C-terminus of the polypeptide linked, or which is reactive with the multifunctional cross-linking agent. In general, polymers bearing such reactive groups are known for the preparation of immobilized proteins. In order to use such chemistries here, one should employ a water soluble polymer otherwise derivatized in the same fashion as

insoluble polymers heretofore employed for protein immobilization. Cyanogen bromide activation is a particularly useful procedure to employ in crosslinking polysaccharides.

"Water soluble" in reference to the starting polymer means that the polymer or its reactive intermediate used for conjugation is sufficiently water soluble to participate in a derivatization reaction.

5 "Water soluble" in reference to the polymer conjugate means that the conjugate is soluble in physiological fluids such as blood.

The degree of substitution with such a polymer will vary depending upon the number of reactive sites on the protein, whether all or a fragment of the protein is used, whether the protein is a fusion with a heterologous protein (e.g. an OB-immunoglobulin chimera), the molecular weight, hydrophilicity and other characteristics of the polymer, and the particular protein derivatization sites chosen. In general, the conjugate contains about from 1 to 10 polymer molecules, while any heterologous sequence may be substituted with an essentially unlimited number of polymer molecules so long as the desired activity is not significantly adversely affected. The optimal degree of cross-linking is easily determined by an experimental matrix in which the time, temperature and other reaction conditions are varied to change the degree of substitution, after which the ability of the conjugates to function in the desired fashion is determined.

The polymer, e.g. PEG, is cross-linked by a wide variety of methods known *per se* for the covalent modification of proteins with nonproteinaceous polymers such as PEG. Certain of these methods, however, are not preferred for the purposes herein. Cyanuronic chloride chemistry leads to many side reactions, including protein cross-linking. In addition, it may be particularly likely to lead to inactivation of proteins containing 20 sulfhydryl groups. Carbonyl diimidazole chemistry (Beauchamp *et al.*, *Anal Biochem.* 131:25-33 (1983)) requires high pH (>8.5), which can inactivate proteins. Moreover, since the "activated PEG" intermediate can react with water, a very large molar excess of "activated PEG" over protein is required. The high concentrations of PEG required for the carbonyl diimidazole chemistry also led to problems in purification, as both gel filtration chromatography and hydrophilic interaction chromatography are adversely affected. In addition, the high 25 concentrations of "activated PEG" may precipitate protein, a problem that *per se* has been noted previously (Davis, U.S. Patent No. 4,179,337). On the other hand, aldehyde chemistry (Royer, U.S. Patent No. 4,002,531) is more efficient since it requires only a 40-fold molar excess of PEG and a 1-2 hr incubation. However, the manganese dioxide suggested by Royer for preparation of the PEG aldehyde is problematic "because of the pronounced tendency of PEG to form complexes with metal-based oxidizing agents" (Harris *et al.*, *J. Polym. Sci. Polym. Chem. Ed.* 22:341-52 (1984)). The use of a Moffatt oxidation, utilizing DMSO and acetic anhydride, obviates this problem. In addition, the sodium borohydride suggested by Royer must be used at high pH and has a significant tendency to reduce disulfide bonds. In contrast, sodium cyanoborohydride, which is effective at neutral pH and has very little tendency to reduce disulfide bonds is preferred.

Functionalized PEG polymers to modify the OB protein or OB-immunoglobulin chimeras of the present 35 invention are available from Shearwater Polymers, Inc. (Huntsville, AL). Such commercially available PEG derivatives include, but are not limited to, amino-PEG, PEG amino acid esters, PEG-hydrazide, PEG-thiol, PEG-succinate, carboxymethylated PEG, PEG-propionic acid, PEG amino acids, PEG succinimidyl succinate, PEG succinimidyl propionate, succinimidyl ester of carboxymethylated PEG, succinimidyl carbonate of PEG, succinimidyl esters of amino acid PEGs, PEG-oxycarbonylimidazole, PEG-nitrophenyl carbonate, PEG tresylate,

PEG-glycidyl ether, PEG-aldehyde, PEG vinylsulfone, PEG-maleimide, PEG-orthopyridyl-disulfide, heterofunctional PEGs, PEG vinyl derivatives, PEG silanes, and PEG phospholides. The reaction conditions for coupling these PEG derivatives will vary depending on the protein, the desired degree of PEGylation, and the PEG derivative utilized. Some factors involved in the choice of PEG derivatives include: the desired point
5 of attachment (lysine or cysteine), hydrolytic stability and reactivity of the derivatives, stability, toxicity and antigenicity of the linkage, suitability for analysis, etc. Specific instructions for the use of any particular derivative are available from the manufacturer.

The long half-life conjugates of this invention are separated from the unreacted starting materials by gel filtration. Heterologous species of the conjugates are purified from one another in the same fashion. The
10 polymer also may be water-insoluble, as a hydrophilic gel.

The conjugates may also be purified by ion-exchange chromatography. The chemistry of many of the electrophilically activated PEG's results in a reduction of amino group charge of the PEGylated product. Thus, high resolution ion exchange chromatography can be used to separate the free and conjugated proteins, and to resolve species with different levels of PEGylation. In fact, the resolution of different species (e.g. containing
15 one or two PEG residues) is also possible due to the difference in the ionic properties of the unreacted amino acids.

B. Therapeutic Uses for the WSX Receptor

The WSX receptor and WSX receptor gene are believed to find therapeutic use for administration to a mammal in the treatment of diseases characterized by a decrease in hematopoietic cells. Examples of these
20 diseases include: anemia (including macrocytic and aplastic anemia); thrombocytopenia; hypoplasia; disseminated intravascular coagulation (DIC); myelodysplasia; immune (autoimmune) thrombocytopenic purpura (ITP); and HIV induced ITP. Additionally, these WSX receptor molecules may be useful in treating myeloproliferative thrombocytotic diseases as well as thrombocytosis from inflammatory conditions and in iron deficiency. WSX receptor polypeptide and WSX receptor gene which lead to an increase in hematopoietic cell
25 proliferation may also be used to enhance repopulation of mature blood cell lineages in cells having undergone chemo- or radiation therapy or bone marrow transplantation therapy. Generally, the WSX receptor molecules are expected to lead to an enhancement of the proliferation and/or differentiation (but especially proliferation) of primitive hematopoietic cells. Other potential therapeutic applications for WSX receptor and WSX receptor gene include the treatment of obesity and diabetes and for promoting kidney, liver and lung growth and/or repair
30 (e.g. in renal failure). WSX receptor can also be used to treat obesity-related conditions, such as type II adult onset diabetes, infertility, hypercholesterolemia, hyperlipidemia, cardiovascular disease and hypertension.

The WSX receptor may be administered alone or in combination with cytokines (such as OB protein), growth factors or antibodies in the above-identified clinical situations. This may facilitate an effective lowering of the dose of WSX receptor. Suitable dosages for such additional molecules will be discussed below.

35 Administration of WSX receptor to a mammal having depressed levels of endogenous WSX receptor or a defective WSX receptor gene is contemplated, preferably in the situation where such depressed levels lead to a pathological disorder, or where there is lack of activation of the WSX receptor. In these embodiments where the full length WSX receptor is to be administered to the patient, it is contemplated that the gene encoding the receptor may be administered to the patient via gene therapy technology.

In gene therapy applications, genes are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. (Zamecnik *et al.*, *Proc. Natl. Acad. Sci. USA*, 83:4143-4146 (1986)). The oligonucleotides can be modified to enhance their uptake, *e.g.*, by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau *et al.*, *Trends in Biotechnology* 11:205-210 (1993)). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.* 262:4429-4432 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256:808-813 (1992).

The invention also provides antagonists of WSX receptor activation (*e.g.* WSX receptor ECD, WSX receptor immunoadhesins and WSX receptor antisense nucleic acid; neutralizing antibodies and uses thereof are discussed in section E below). Administration of WSX receptor antagonist to a mammal having increased or excessive levels of endogenous WSX receptor activation is contemplated, preferably in the situation where such levels of WSX receptor activation lead to a pathological disorder.

In one embodiment, WSX receptor antagonist molecules may be used to bind endogenous ligand in the body, thereby causing desensitized WSX receptors to become responsive to WSX ligand, especially when the levels of WSX ligand in the serum exceed normal physiological levels. Also, it may be beneficial to bind endogenous WSX ligand which is activating undesired cellular responses (such as proliferation of tumor cells). Potential therapeutic applications for WSX antagonists include for example, treatment of metabolic disorders (*e.g.*, anorexia, cachexia, steroid-induced truncal obesity and other wasting diseases characterized by loss of appetite, diminished food intake or body weight loss), stem cell tumors and other tumors which express WSX receptor.

Pharmaceutical compositions of the WSX receptor ECD may further include a WSX ligand. Such dual compositions may be beneficial where it is therapeutically useful to prolong half-life of WSX ligand, and/or activate endogenous WSX receptor directly as a heterotrimeric complex.

Therapeutic formulations of WSX receptor are prepared for storage by mixing WSX receptor having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A., Ed., (1980)), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as Tween, Plurionics™ or polyethylene glycol (PEG).

The WSX receptor also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, *supra*.

WSX receptor to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. WSX receptor ordinarily will be stored in lyophilized form or in solution.

Therapeutic WSX receptor compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of WSX receptor administration is in accord with known methods, *e.g.*, those routes set forth above for specific indications, as well as the general routes of injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional means, or sustained release systems as noted below. WSX receptor is administered continuously by infusion or by bolus injection. Generally, where the disorder permits, one should formulate and dose the WSX receptor for site-specific delivery.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (*e.g.*, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, *J. Biomed. Mater. Res.* 15:167-277 (1981) and Langer, *Chem. Tech.* 12:98-105 (1982) or poly(vinylalcohol)), polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and γ ethyl-L-glutamate (Sidman *et al.*, *Biopolymers* 22:547-556 (1983)), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron

DepotTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release WSX receptor compositions also include liposomally entrapped WSX receptor. Liposomes containing WSX receptor are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688-3692 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal WSX receptor therapy.

When applied topically, the WSX receptor is suitably combined with other ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except that they must be physiologically acceptable and efficacious for their intended administration, and cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen. The compositions also may be impregnated into transdermal patches, plasters, and bandages, preferably in liquid or semi-liquid form.

For obtaining a gel formulation, the WSX receptor formulated in a liquid composition may be mixed with an effective amount of a water-soluble polysaccharide or synthetic polymer such as PEG to form a gel of the proper viscosity to be applied topically. The polysaccharide that may be used includes, for example, cellulose derivatives such as etherified cellulose derivatives, including alkyl celluloses, hydroxyalkyl celluloses, and alkylhydroxyalkyl celluloses, for example, methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; starch and fractionated starch; agar; alginic acid and alginates; gum arabic; pullulan; agarose; carrageenan; dextrans; fructans; inulin; mannans; xylans; arabinans; chitosans; glycogens; glucans; and synthetic biopolymers; as well as gums such as xanthan gum; guar gum; locust bean gum; gum arabic; tragacanth gum; and karaya gum; and derivatives and mixtures thereof. The preferred gelling agent herein is one that is inert to biological systems, nontoxic, simple to prepare, and not too runny or viscous, and will not destabilize the WSX receptor held within it.

Preferably the polysaccharide is an etherified cellulose derivative, more preferably one that is well defined, purified, and listed in USP, *e.g.*, methylcellulose and the hydroxyalkyl cellulose derivatives, such as hydroxypropyl cellulose, hydroxyethyl cellulose, and hydroxypropyl methylcellulose. Most preferred herein is methylcellulose.

The polyethylene glycol useful for gelling is typically a mixture of low and high molecular weight PEGs to obtain the proper viscosity. For example, a mixture of a PEG of molecular weight 400-600 with one of molecular weight 1500 would be effective for this purpose when mixed in the proper ratio to obtain a paste.

The term "water soluble" as applied to the polysaccharides and PEGs is meant to include colloidal solutions and dispersions. In general, the solubility of the cellulose derivatives is determined by the degree of substitution of ether groups, and the stabilizing derivatives useful herein should have a sufficient quantity of such ether groups per anhydroglucose unit in the cellulose chain to render the derivatives water soluble. A degree of ether substitution of at least 0.35 ether groups per anhydroglucose unit is generally sufficient. Additionally, the cellulose derivatives may be in the form of alkali metal salts, for example, the Li, Na, K, or Cs salts.

If methylcellulose is employed in the gel, preferably it comprises about 2-5%, more preferably about 3%, of the gel and the WSX receptor is present in an amount of about 300-1000 mg per ml of gel.

An effective amount of WSX receptor to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the WSX receptor until a dosage is reached that achieves the desired effect. A typical daily dosage for systemic treatment might range from about 1 µg/kg to up to 10 mg/kg or more, depending on the factors mentioned above. As an alternative general proposition, the WSX receptor is formulated and delivered to the target site or tissue at a dosage capable of establishing in the tissue a WSX receptor level greater than about 0.1 ng/cc up to a maximum dose that is efficacious but not unduly toxic. This intra-tissue concentration should be maintained if possible by continuous infusion, sustained release, topical application, or injection at empirically determined frequencies. The progress of this therapy is easily monitored by conventional assays.

C. Non-Therapeutic Uses for the WSX Receptor

WSX receptor nucleic acid is useful for the preparation of WSX receptor polypeptide by recombinant techniques exemplified herein which can then be used for production of anti-WSX receptor antibodies having various utilities described below.

The WSX receptor (polypeptide or nucleic acid) can be used to induce proliferation and/or differentiation of cells *in vitro*. In particular, it is contemplated that this molecule may be used to induce proliferation of stem cell/progenitor cell populations (e.g. CD34+ cell populations obtained as described in Example 8 below). These cells which are to be grown *ex vivo* may simultaneously be exposed to other known growth factors or cytokines, such as those described herein. This results in proliferation and/or differentiation of the cells having the WSX receptor.

In yet another aspect of the invention, the WSX receptor may be used for affinity purification of WSX ligand. Briefly, this technique involves: (a) contacting a source of WSX ligand with an immobilized WSX receptor under conditions whereby the WSX ligand to be purified is selectively adsorbed onto the immobilized receptor; (b) washing the immobilized WSX receptor and its support to remove non-adsorbed material; and (c) eluting the WSX ligand molecules from the immobilized WSX receptor to which they are adsorbed with an elution buffer. In a particularly preferred embodiment of affinity purification, WSX receptor is covalently attaching to an inert and porous matrix (e.g., agarose reacted with cyanogen bromide). Especially preferred is

a WSX receptor immunoadhesin immobilized on a protein A column. A solution containing WSX ligand is then passed through the chromatographic material. The WSX ligand adsorbs to the column and is subsequently released by changing the elution conditions (*e.g.* by changing pH or ionic strength).

The WSX receptor may be used for competitive screening of potential agonists or antagonists for binding to the WSX receptor. Such agonists or antagonists may constitute potential therapeutics for treating conditions characterized by insufficient or excessive WSX receptor activation, respectively.

The preferred technique for identifying molecules which bind to the WSX receptor utilizes a chimeric receptor (*e.g.*, epitope tagged WSX receptor or WSX receptor immunoadhesin) attached to a solid phase, such as the well of an assay plate. Binding of molecules which are optionally labelled (*e.g.*, radiolabelled) to the immobilized receptor can be evaluated.

To identify WSX receptor agonists or antagonists, the thymidine incorporation assay can be used. For screening for antagonists, the WSX receptor can be exposed to a WSX ligand followed by the putative antagonist, or the WSX ligand and antagonist can be added to the WSX receptor simultaneously, and the ability of the antagonist to block receptor activation can be evaluated.

The WSX receptor polypeptides are also useful as molecular weight markers. To use a WSX receptor polypeptide as a molecular weight marker, gel filtration chromatography or SDS-PAGE, for example, will be used to separate protein(s) for which it is desired to determine their molecular weight(s) in substantially the normal way. The WSX receptor and other molecular weight markers will be used as standards to provide a range of molecular weights. For example, phosphorylase b (mw = 97,400), bovine serum albumin (mw = 68,000), ovalbumin (mw = 46,000), WSX receptor (mw = 44,800), trypsin inhibitor (mw = 20,100), and lysozyme (mw = 14,400) can be used as mw markers. The other molecular weight markers mentioned here can be purchased commercially from Amersham Corporation, Arlington Heights, IL. The molecular weight markers are generally labeled to facilitate detection thereof. For example, the markers may be biotinylated and following separation can be incubated with streptavidin-horseradish peroxidase so that the various markers can be detected by light detection.

The purified WSX receptor, and the nucleic acid encoding it, may also be sold as reagents for mechanism studies of WSX receptor and its ligands, to study the role of the WSX receptor and WSX ligand in normal growth and development, as well as abnormal growth and development, *e.g.* in malignancies.

WSX receptor variants are useful as standards or controls in assays for the WSX receptor for example ELISA, RIA, or RRA, provided that they are recognized by the analytical system employed, *e.g.*, an anti-WSX receptor antibody.

D. WSX Receptor Antibody Preparation

1. Polyclonal antibodies

Polyclonal antibodies are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. In that the preferred epitope is in the ECD of the WSX receptor, it is desirable to use WSX receptor ECD or a molecule comprising the ECD (*e.g.*, WSX receptor immunoadhesin) as the antigen for generation of polyclonal and monoclonal antibodies. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or

derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining 1 mg or 1 μg of the peptide or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

2. Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (Cabilly *et al.*, *supra*).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

- 5 The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.* 107:220 (1980).

- After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

- DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells.
- 15 Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.* 5:256-262 (1993) and Plückthun, *Immunol. Revs.* 130:151-188 (1992).

- In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature* 348:552-554 (1990). Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Mark *et al.*, *BioTechnology* 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

- 30 The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (Cabilly *et al.*, *supra*; Morrison, *et al.*, *Proc. Nat. Acad. Sci. USA* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

- Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.
- 35 Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using

a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

3. Humanized and human antibodies

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-327 (1988); Verhoeven *et al.*, *Science* 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly *et al.*, *supra*), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.* 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.* 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA* 89:4285 (1992); Presta *et al.*, *J. Immunol.* 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody

production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90:2551 (1993); Jakobovits *et al.*, *Nature* 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.* 7:33 (1993). Human antibodies can also be produced in phage-display libraries (Hoogenboom *et al.*, *J. Mol. Biol.* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.* 222:581 (1991)).

4. Bispecific antibodies

Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different antigens. BsAbs can be used as tumor targeting or imaging agents and can be used to target enzymes or toxins to a cell possessing the WSX receptor. Such antibodies can be derived from full length antibodies or antibody fragments (e.g. F(ab')₂ bispecific antibodies). In accordance with the present invention, the BsAb may possess one arm which binds the WSX receptor and another arm which binds to a cytokine or another cytokine receptor (or a subunit thereof) such as the receptors for TPO, EPO, G-CSF, IL-4, IL-7, GH, PRL; the α or β subunits of the IL-3, GM-CSF, IL-5, IL-6, LIF, OSM and CNTF receptors; or the α , β or γ subunits of the IL-2 receptor complex. For example, the BsAb may bind both WSX receptor and gp130.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature* 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991).

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published March 3, 1994.

For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology* 121:210 (1986).

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. The following techniques can also be used for the production of bivalent antibody fragments which are not necessarily bispecific. According to these techniques, Fab'-SH fragments can be recovered from *E. coli*, which can be chemically coupled to form bivalent antibodies. Shalaby *et al.*, *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized BsAb F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the BsAb. The BsAb thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets. See also Rodrigues *et al.*, *Int. J. Cancers* (Suppl.) 7:45-50 (1992).

Various techniques for making and isolating bivalent antibody fragments directly from recombinant cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making BsAb fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making BsAb fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.* 152:5368 (1994).

5. Antibody Screening

It may be desirable to select antibodies with a strong binding affinity for the WSX receptor. Antibody affinities may be determined by saturation binding; enzyme-linked immunoabsorbent assay (ELISA); and competition assays (e.g. RIA's), for example. The antibody with a strong binding affinity may bind the WSX receptor with a binding affinity (K_d) value of no more than about 1 x 10⁻⁷ M, preferably no more than about 1 x 10⁻⁸ M and most preferably no more than about 1 x 10⁻⁹ M (e.g. to about 1 x 10⁻¹² M).

In another embodiment, one may screen for an antibody which binds a WSX receptor epitope of interest. For example, an antibody which binds to the epitope bound by antibody 2D7, 1G4, 1E11 or 1C11 (see Example 13) or antibody clone #3, #4 or #17 (see Example 14) can be identified. To screen for antibodies which bind to the epitope on WSX receptor bound by an antibody of interest (e.g., those which block binding of any one

of the above antibodies to WSX receptor), a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping, e.g. as described in Champe *et al.*, *J. Biol. Chem.* 270:1388-1394 (1995), can be performed to determine whether the antibody binds an epitope of interest.

5 In one particularly preferred embodiment of the invention, agonist antibodies are selected. Various methods for selecting agonist antibodies are available. In one embodiment, one evaluates the agonistic properties of the antibody upon binding to a chimeric receptor comprising the WSX receptor extracellular domain in an assay called the kinase receptor activation enzyme linked immunoadsorbent assay (KIRA ELISA) described in WO95/14930 (expressly incorporated herein by reference).

10 To perform the KIRA ELISA, a chimeric receptor comprising the extracellular domain of the WSX receptor and the transmembrane and intracellular domain of Rse receptor (Mark *et al.*, *Journal of Biological Chemistry* 269(14):10720-10728 (1994)) with a carboxyl-terminal herpes simplex virus glycoprotein D (gD) tag is produced and dp12.CHO cells are transformed therewith as described in Example 4 of WO95/14930.

The WSX/Rse.gD transformed dp12.CHO cells are seeded (3×10^4 per well) in the wells of a flat-bottom-96 well culture plate in 100 μ l media and cultured overnight at 37°C in 5% CO₂. The following morning the well supernatants are removed and various concentrations of the antibody are added to separate wells. The cells are stimulated at 37°C for 30 min., the well supernatants are decanted. To lyse the cells and solubilize the chimeric receptors, 100 μ l of lysis buffer is added to each well. The plate is then agitated gently on a plate shaker (Bellco Instruments, Vineland, NJ) for 60 min. at room temperature.

20 While the cells are being solubilized, an ELISA microtiter plate (Nunc Maxisorp, Inter Med, Denmark) coated overnight at 4°C with the 5B6 monoclonal anti-gD antibody (5.0 μ g/ml in 50 mM carbonate buffer, pH 9.6, 100 μ l/well) is decanted and blocked with 150 μ l/well of Block Buffer for 60 min. at room temperature. After 60 minutes, the anti-gD 5B6 coated plate is washed 6 times with wash buffer (PBS containing 0.05 % TWEEN 20™ and 0.01 % thimerosal).

25 The lysate containing solubilized WSX/Rse.gD from the cell-culture microtiter well is transferred (85 μ l/well) to anti-gD 5B6 coated and blocked ELISA well and is incubated for 2 h at room temperature. The unbound WSX/Rse.gD is removed by washing with wash buffer and 100 μ l of biotinylated 4G10 (anti-phosphotyrosine) diluted 1:18000 in dilution buffer (PBS containing 0.5 % BSA, 0.05 % Tween-20, 5 mM EDTA, and 0.01 % thimerosal), i.e. 56 ng/ml is added to each well. After incubation for 2 h at room temperature the plate is washed and HRPO-conjugated streptavidin (Zymed Laboratories, S. San Francisco, CA) is added to each well. The plate is incubated for 30 minutes at room temperature with gentle agitation. The free avidin-conjugate is washed away and 100 μ l freshly prepared substrate solution (tetramethyl benzidine (TMB); 2-component substrate kit; Kirkegaard and Perry, Gaithersburg, MD) is added to each well. The reaction is allowed to proceed for 10 minutes, after which the color development is stopped by the addition of 100 μ l/well
35 1.0 M H₃PO₄. The absorbance at 450 nm is read with a reference wavelength of 650 nm (ABS_{450/650}), using a *vmax* plate reader (Molecular Devices, Palo Alto, CA) controlled with a Macintosh Centris 650 (Apple Computers, Cupertino, CA) and DeltaSoft software (BioMetallics, Inc, Princeton, NJ).

Those antibodies which have an IC₅₀ in the KIRA ELISA of about 0.5 µg/ml or less (e.g. from about 0.5 µg/ml to about 0.001 µg/ml), preferably about 0.2 µg/ml or less and most preferably about 0.1 µg/ml or less are preferred agonists.

In another embodiment, one screens for antibodies which activate downstream signaling molecules for OB protein. For example, the ability of the antibody to activate Signal Transducers and Activators of Transcription (STATs) can be assessed. The agonist antibody of interest may stimulate formation of STAT-1 and STAT-3 complexes, for example. To screen for such antibodies, the assay described in Rosenblum *et al. Endocrinology* 137(11):5178-5181 (1996) may be performed.

Alternatively, an antibody which stimulates proliferation and/or differentiation of hematopoietic cells can be selected. For example, the hematopoiesis assays of Example 10 below can be performed. For example, murine fetal liver fLASK stem cells may be isolated from the midgestational fetal liver as described in Zeigler *et al., Blood* 84:2422-2430 (1994) and studied in stem cell suspension culture or methylcellulose assays. For the stem cell suspension cultures, twenty thousand of the fLASK cells are seeded in individual wells in a 12 well format in DMEM 4.5/F12 media supplemented with 10% heat inactivated fetal calf serum (Hyclone, Logan, UT) and L-glutamine. Growth factors are added at the following concentrations: kit ligand (KL) at 25 ng/mL, interleukin-3 (IL-3) at 25 ng/mL, interleukin-6 (IL-6) at 50 ng/mL, G-CSF at 100 ng/mL, GM-CSF at 100 ng/mL, EPO at 2U/mL, interleukin-7 (IL-7) at 100 ng/mL (all growth factors from R and D Systems, Minneapolis, MN). The agonist antibody is then added and the ability of the antibody to expand the fLASK cells grown in suspension culture is assessed. Methylcellulose assays are performed as previously described (Zeiger *et al., supra*). Briefly, methylcellulose colony assays are performed using "complete" methylcellulose or pre-B methylcellulose medium (Stem Cell Technologies, Vancouver, British Columbia, Canada) with the addition of 25 ng/mL KL (R and D Systems, Minneapolis, MN). Cytospin analyses of the resultant colonies are performed as previously described in Zeigler *et al.* The ability of the agonist antibody to augment myeloid, lymphoid and erythroid colony formation is assessed. Also, the effect of the agonist antibody on the murine bone marrow stem cell population; Lin^{lo}Sca⁺ may be evaluated.

One may select an agonist antibody which induces a statistically significant decrease in body weight and/or fat-depot weight and/or food intake in an obese mammal (e.g. in an *ob/ob* mouse). Methods for screening for such molecules are described in Levin *et al. Proc. Natl. Acad. Sci. USA* 93:1726-1730 (1996), for example. Preferred agonist antibodies are those which exert adipose-reducing effects in an obese mammal, such as the *ob/ob* mouse, which are in excess of those induced by reductions in food intake.

The antibody of interest herein may have the hypervariable region residues of one of the antibodies in Examples 13 and 14. Also, the invention encompasses "affinity matured" forms of these antibodies in which hypervariable region residues of these antibodies have been modified. Such affinity matured antibodies will preferably have a biological activity which is the same as or better than that of the original antibody. The affinity matured antibody may have from about 1-10, e.g. 5-10 deletions, insertions or substitutions (but preferably substitutions) in the hypervariable regions thereof. One useful procedure for generating affinity matured antibodies is called "alanine scanning mutagenesis" (Cunningham and Wells *Science* 244:1081-1085 (1989)). Here, one or more of the hypervariable region residue(s) are replaced by alanine or polyalanine residue(s) to affect the interaction of the amino acids with the WSX receptor. Those hypervariable region residue(s)

demonstrating functional sensitivity to substitution are then refined by introducing further or other mutations at or for the sites of substitution. The ala-mutants produced this way are screened for their biological activity as described herein. Another procedure is affinity maturation using phage display (Hawkins *et al.* *J. Mol. Biol.* 254:889-896 (1992) and Lowman *et al.* *Biochemistry* 30(45):10832-10837 (1991)). Briefly, several

5 hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody mutants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed mutants are then screened for their biological activity (*e.g.* binding affinity).

6. Antibody Modifications

10 It may be desirable to tailor the antibody for various applications. Exemplary antibody modifications are described here.

In certain embodiments of the invention, it may be desirable to use an antibody fragment, rather than an intact antibody. In this case, it may be desirable to modify the antibody fragment in order to increase its serum half-life. This may be achieved, for example, by incorporation of a salvage receptor binding epitope into the

15 antibody fragment. See WO96/32478 published October 17, 1996. Alternatively, the antibody may be conjugated to a nonproteinaceous polymer, such as those described above for the production of long half-life derivatives of OB protein.

Where the antibody is to be used to treat cancer for example, various modifications of the antibody (*e.g.* of a neutralizing antibody) which enhance the effectiveness of the antibody for treating cancer are contemplated

20 herein. For example, it may be desirable to modify the antibody of the invention with respect to effector function. For example cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp. Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.*

25 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* *Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.* *Anti-Cancer Drug Design* 3:219-230 (1989).

The invention also pertains to immunoconjugates comprising the antibody described herein conjugated

30 to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A

35 chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictin, phenomycin, enmycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugate antibodies. Examples include ²¹²Bi, ¹³¹I, ¹³¹In, ⁹⁰Y and ¹⁸⁶Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

The antibody may also be formulated as an immunoliposome. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl. Acad. Sci. USA*, 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.* 81(19):1484 (1989).

The antibody of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β -lactamase useful for converting drugs derivatized with β -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxycetyl or phenylacetyl groups, respectively,

into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

5 The enzymes of this invention can be covalently bound to the antibody mutant by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

10 In other embodiments, the antibody can be covalently modified, with exemplary such modifications described above.

E. Therapeutic Uses for WSX Receptor Ligands and Antibodies

 The WSX ligands (*e.g.* OB protein and anti-WSX receptor agonist antibodies) of the present invention are useful, in one embodiment, for weight reduction, and specifically, in the treatment of obesity, bulimia and
15 other disorders associated with the abnormal expression or function of the OB and/or WSX receptor genes, other metabolic disorders such as diabetes, for reducing excessive levels of insulin in human patients (*e.g.* to restore or improve the insulin-sensitivity of such patients). Thus, these molecules can be used to treat a patient suffering from excessive food consumption and related pathological conditions such as type II adult onset diabetes, infertility (Chehab *et al.* *Nature Genetics* 12:318-320 (1996)), hypercholesterolemia, hyperlipidemia,
20 cardiovascular diseases, arteriosclerosis, polycystic ovarian disease, osteoarthritis, dermatological disorders, insulin resistance, hypertriglyceridemia, cancer, cholelithiasis and hypertension.

 In addition, the WSX ligands can be used for the treatment of kidney ailments, hypertension, and lung dysfunctions, such as emphysema.

 In a further embodiment, the WSX ligands (such as agonist WSX receptor antibodies) of the present
25 invention can be used to enhance repopulation of mature blood cell lineages in mammals having undergone chemo- or radiation therapy or bone marrow transplantation therapy. Generally, the ligands will act via an enhancement of the proliferation and/or differentiation (but especially proliferation) of primitive hematopoietic cells. The ligands may similarly be useful for treating diseases characterized by a decrease in blood cells. Examples of these diseases include: anemia (including macrocytic and aplastic anemia); thrombocytopenia;
30 hypoplasia; immune (autoimmune) thrombocytopenic purpura (ITP); and HIV induced ITP. Also, the ligands may be used to treat a patient having suffered a hemorrhage. WSX ligands may also be used to treat metabolic disorders such as obesity and diabetes mellitus, or to promote kidney, liver or lung growth and/or repair (*e.g.* in renal failure).

 The WSX receptor ligands and antibodies may be administered alone or in concert with one or more
35 cytokines. Furthermore, as an alternative to administration of the WSX ligand protein, gene therapy techniques (discussed in the section above entitled "Therapeutic Uses for the WSX Receptor") are also contemplated herein.

 Potential therapeutic applications for WSX receptor neutralizing antibodies include the treatment of metabolic disorders (such as cachexia, anorexia and other wasting diseases characterized by loss of appetite,

diminished food intake or body weight loss), stem cell tumors and other tumors at sites of WSX receptor expression, especially those tumors characterized by overexpression of WSX receptor.

For therapeutic applications, the WSX receptor ligands and antibodies of the invention are administered to a mammal, preferably a human, in a physiologically acceptable dosage form, including those that may be administered to a human intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intra-cerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The WSX receptor ligands and antibodies also are suitably administered by intratumoral, peritumoral, intralesional, or perilesional routes or to the lymph, to exert local as well as systemic therapeutic effects.

Such dosage forms encompass physiologically acceptable carriers that are inherently non-toxic and non-therapeutic. Examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and PEG. Carriers for topical or gel-based forms of WSX receptor antibodies include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, PEG, and wood wax alcohols. For all administrations, conventional depot forms are suitably used. Such forms include, for example, microcapsules, nano-capsules, liposomes, plasters, inhalation forms, nose sprays, sublingual tablets, and sustained-release preparations. The WSX receptor ligand or antibody will typically be formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the WSX receptor ligand or antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, *supra* and Langer, *supra*, or poly(vinylalcohol), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate (Sidman *et al.*, *supra*), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron DepotTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated WSX receptor antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release WSX receptor ligand or antibody compositions also include liposomally entrapped antibodies. Liposomes containing the WSX receptor ligand or antibody are prepared by methods known in the

art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Ordinarily, the liposomes are the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol.% cholesterol, the selected proportion being adjusted for the optimal WSX receptor ligand or antibody therapy.

5 Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

For the prevention or treatment of disease, the appropriate dosage of WSX receptor ligand or antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibodies are administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the WSX receptor ligand or antibody, and the discretion of the attending physician. The

10 WSX receptor ligand or antibody is suitably administered to the patient at one time or over a series of treatments.

Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg of WSX receptor ligand or antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 µg/kg (*e.g.* 1-50 µg/kg) or more, depending on the factors mentioned above. For example, the dose may be

15 the same as that for other cytokines such as G-CSF, GM-CSF and EPO. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

When one or more cytokines are co-administered with the WSX receptor ligand, lesser doses of the

20 WSX ligand may be employed. Suitable doses of a cytokine are from about 1 µg/kg to about 15mg/kg of cytokine. A typical daily dosage of the cytokine might range from about 1 µg/kg to 100 µg/kg (*e.g.* 1-50 µg/kg) or more. For example, the dose may be the same as that for other cytokines such as G-CSF, GM-CSF and EPO. The cytokine(s) may be administered prior to, simultaneously with, or following administration of the WSX ligand. The cytokine(s) and WSX ligand may be combined to form a pharmaceutically composition for

25 simultaneous administration to the mammal. In certain embodiments, the amounts of WSX ligand and cytokine are such that a synergistic repopulation of blood cells (or synergistic increase in proliferation and/or differentiation of hematopoietic cells) occurs in the mammal upon administration of the WSX ligand and cytokine thereto. In other words, the coordinated action of the two or more agents (*i.e.* the WSX ligand and cytokine(s)) with respect to repopulation of blood cells (or proliferation/differentiation of hematopoietic cells)

30 is greater than the sum of the individual effects of these molecules.

For treating obesity and associated pathological conditions, the WSX ligand may be administered in combination with other treatments for combatting or preventing obesity. Substances useful for this purpose include, *e.g.*, hormones (catecholamines, glucagon, ACTH); clofibrate; halogenate; cinchocaine; chlorpromazine; appetite-suppressing drugs acting on noradrenergic neurotransmitters such as mazindol and derivatives of

35 phenethylamine, *e.g.*, phenylpropanolamine, diethylpropion, phentermine, phendimetrazine, benzphetamine, amphetamine, methamphetamine, and phenmetrazine; drugs acting on serotonin neurotransmitters such as fenfluramine, tryptophan, 5-hydroxytryptophan, fluoxetine, and sertraline; centrally active drugs such as naloxone, neuropeptide-Y, galanin, corticotropin-releasing hormone, and cholecystokinin; a cholinergic agonist such as pyridostigmine; a sphingolipid such as a lysosphingolipid or derivative thereof (EP 321,287 published

June 21, 1989); thermogenic drugs such as thyroid hormone, ephedrine, beta-adrenergic agonists; drugs affecting the gastrointestinal tract such as enzyme inhibitors, *e.g.*, tetrahydrolipostatin, indigestible food such as sucrose polyester, and inhibitors of gastric emptying such as threo-chlorocitric acid or its derivatives; β -adrenergic agonist such as isoproterenol and yohimbine; aminophylline to increase the β -adrenergic-like effects of yohimbine, an α_2 -adrenergic blocking drug such as clonidine alone or in combination with a growth hormone releasing peptide (U.S. Pat. No. 5,120,713 issued June 9, 1992); drugs that interfere with intestinal absorption such as biguanides such as metformin and phenformin; bulk fillers such as methylcellulose; metabolic blocking drugs such as hydroxycitrate; progesterone; cholecystokinin agonists; small molecules that mimic ketoacids; agonists to corticotropin-releasing hormone; an ergot-related prolactin-inhibiting compound for reducing body fat stores (U.S. Pat. No. 4,783,469 issued November 8, 1988); beta-3-agonists; bromocriptine; antagonists to opioid peptides; antagonists to neuropeptide Y; glucocorticoid receptor antagonists; growth hormone agonists; combinations thereof; *etc.* This includes all drugs described by Bray and Greenway, *Clinics in Endocrinol. and Metabol.*, 5:455 (1976).

These adjunctive agents may be administered at the same time as, before, or after the administration of WSX ligand and can be administered by the same or a different administration route than the WSX ligand.

The WSX ligand treatment may occur without, or may be imposed with, a dietary restriction such as a limit in daily food or calorie intake, as is desired for the individual patient.

F. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the conditions described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the WSX ligand. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container holding a cytokine for co-administration with the WSX ligand. Further container(s) may be provided with the article of manufacture which may hold, for example, a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution or dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

G. Non-Therapeutic Uses for WSX Receptor Ligands and Antibodies

WSX receptor ligands and antibodies may be used for detection of and/or enrichment of hematopoietic stem cell/progenitor cell populations in a similar manner to that in which CD34 antibodies are presently used. For stem cell enrichment, the WSX receptor antibodies may be utilized in the techniques known in the art such as immune panning, flow cytometry or immunomagnetic beads.

In accordance with the *in vitro* application of the WSX ligands, cells comprising the WSX receptor are provided and placed in a cell culture medium. Examples of such WSX-receptor-containing cells include hematopoietic progenitor cells, such as CD34+ cells.

Suitable tissue culture media are well known to persons skilled in the art and include, but are not limited to, Minimal Essential Medium (MEM), RPMI-1640, and Dulbecco's Modified Eagle's Medium (DMEM). These tissue culture medias are commercially available from Sigma Chemical Company (St. Louis, MO) and GIBCO (Grand Island, NY). The cells are then cultured in the cell culture medium under conditions sufficient for the
5 cells to remain viable and grow in the presence of an effective amount of WSX ligand and, optionally, further cytokines and growth factors. The cells can be cultured in a variety of ways, including culturing in a clot, agar, or liquid culture.

The cells are cultured at a physiologically acceptable temperature such as 37°C, for example, in the presence of an effective amount of WSX ligand. The amount of WSX ligand may vary, but preferably is in the
10 range of about 10 ng/ml to about 1mg/ml. The WSX ligand can of course be added to the culture at a dose determined empirically by those in the art without undue experimentation. The concentration of WSX ligand in the culture will depend on various factors, such as the conditions under which the cells and WSX ligand are cultured. The specific temperature and duration of incubation, as well as other culture conditions, can be varied depending on such factors as, *e.g.*, the concentration of the WSX ligand, and the type of cells and medium.

It is contemplated that using WSX ligand to enhance cell proliferation and/or differentiation *in vitro* will be useful in a variety of ways. For instance, hematopoietic cells cultured *in vitro* in the presence of WSX ligand can be infused into a mammal suffering from reduced levels of the cells. Also, the cultured hematopoietic cells may be used for gene transfer for gene therapy applications. Stable *in vitro* cultures can be also used for
15 isolating cell-specific factors and for expression of endogenous or recombinantly introduced proteins in the cell. WSX ligand may also be used to enhance cell survival, proliferation and/or differentiation of cells which support the growth and/or differentiation of other cells in cell culture.

The WSX receptor antibodies of the invention are also useful as affinity purification agents. In this process, the antibodies against WSX receptor are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample
25 containing the WSX receptor to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the WSX receptor, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the WSX receptor from the antibody.

WSX receptor antibodies may also be useful in diagnostic assays for WSX receptor, *e.g.*, detecting its
30 expression in specific cells, tissues, or serum. For diagnostic applications, antibodies typically will be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ³H, ¹⁴C, ³²P, ³⁵S, or ¹²⁵I; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; radioactive isotopic labels, such as, *e.g.*, ¹²⁵I, ³²P, ¹⁴C, or ³H; or an enzyme, such as alkaline
35 phosphatase, beta-galactosidase, or horseradish peroxidase.

Any method known in the art for separately conjugating the polypeptide variant to the detectable moiety may be employed, including those methods described by Hunter *et al.*, *Nature* 144:945 (1962); David *et al.*, *Biochemistry* 13:1014 (1974); Pain *et al.*, *J. Immunol. Meth* 40:219 (1981); and Nygren, *J. Histochem. and Cytochem.* 30:407 (1982).

The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample
 5 analyte for binding with a limited amount of antibody. The amount of WSX receptor in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

10 Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin
 15 antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

H. Deposit of Materials

The following biological materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

20	Deposit Designation	ATCC No.	Deposit Date
	Baf3/WSX E63x7 sort (Baf3 cells expressing human WSX receptor variant 13.2)	ATCC CRL 12015	Jan 10, 1996
	2D7 hybridoma cell line		
25	1G4 hybridoma cell line	ATCC HB-12243	Dec 11, 1996
	1E11 hybridoma cell line		
	1C11 hybridoma cell line		

These deposits were made under the provisions of the Budapest Treaty on the International Recognition
 of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest
 30 Treaty). This assures maintenance of a viable culture for 30 years from the date of deposit. Each of the deposited cultures will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures (a) that access to the culture will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under

37 CFR §1.14 and 35 USC §122, and (b) that all restrictions on the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent.

The assignee of the present application has agreed that if any of the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, it will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited cell lines is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by any culture deposited, since the deposited embodiment is intended as an illustration of one aspect of the invention and any culture that is functionally equivalent is within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustration that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

III. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

The disclosures of all publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

EXAMPLE 1

Cloning of Human WSX Receptor

An oligonucleotide probe designated WSX.6 #1 was synthesized based upon the T73849 EST sequence.

The WSX.6 #1 probe was a 51mer having the following sequence:
5' GTCAGTCTCCCAGTTCAGACTTGTGTGCAGTCTATGCTGTTCAAGGTGCGC - 3' (SEQ ID NO:45).

The radiolabeled WSX.6 #1 probe was used to probe 1.2×10^6 clones from a random and oligo dT primed λ gt10 fetal liver library (Clontech, Palo Alto, CA). Following hybridization at 42°C overnight, the filters were washed at 50°C in 0.5 x SSC and 0.1% NaDodSO₄ (SDS). From the initial screen, 10 clones were selected and upon subsequent screening 5 individual plaque pure clones were isolated. Of these 5 individual clones, four clones designated 1, 5, 6 and 9 were subcloned into pBSSK⁺ (Stratagene) following EcoRI digestion. Sequence analysis revealed clone 5 and clone 9 contained the putative initiation methionine and signal peptide. Clone 6 (designated 6.4) contained the most 3' end sequence and subsequently was used for further screening.

To obtain the full length gene, clone 6.4 (fragment Nsi-Hind III) was radiolabeled and used to screen 1.2×10^6 clones from a λ gt 10 library constructed from a hepatoma Hep3B cell line. This screen resulted in 24 positive clones. Following PCR analysis of the clones using λ gt10 primers (F and R), the four longest clones 12.1, 13.2, 22.3, and 24.3 were isolated. These clones were subcloned into pBSSK⁺ using the EcoRI site, and following examination by restriction enzyme digest, clones 12.1 and 13.2 were submitted for sequencing. DNA sequencing was performed with the Taq dye deoxynucleotide terminator cycle sequencing kit on an automated Applied Biosystems DNA sequencer.

The assembled contiguous sequence from all the isolated clones encoded a consensus amino terminus for the newly identified polypeptide designated the WSX receptor. However, sequence analysis revealed that at least three naturally occurring variants of the WSX receptor exist which have different cytoplasmic regions. These variants appear to be differentially spliced at the lysine residue at position 891. Clone 6.4 stops 5 amino acids after Lys 891. Clone 12.1 is different from 13.2 and 6.4 following Lys 891 and encodes a putative box 2 region which is distinct from that encoded by clone 13.2. Clone 13.2 contains a potential box 1 region and following Lys 891 encodes putative box 2 and box 3 motifs. See, Baumann *et al.*, *Mol. Cell. Biol.* 14(1):138-146 (1994).

The full length WSX gene based on the clone 13.2 cytoplasmic region putatively encodes an 1165 amino acid transmembrane protein. The 841 amino acid extracellular domain (ECD) contains two WSXWS domains. The ECD is followed by a 24 amino acid transmembrane domain and a 300 amino acid cytoplasmic region.

EXAMPLE 2

WSX Receptor Immunoadhesin

Using polymerase chain amplification, a WSX receptor immunoadhesin was created by engineering an in-frame fusion of the WSX receptor gene extracellular domain (WSX.ECD) with human CH2CH3(Fc)IgG (Bennett *et al.*, *J. Biol. Chem.* 266(34):23060-23067 (1991)) at the C terminus of the ECD and cloned into pBSSK⁺ (Stratagene). For expression, the WSX-Fc was excised with ClaI and BstEII and ligated into the pRK5.HuIF.grbhlIgG Genenase I vector (Beck *et al.*, *Molecular Immunology* 31(17):1335-1344 (1994)), to create the plasmid pRK5.WSX-IgG Genenase I. This plasmid was transiently transfected into 293 cells using standard calcium phosphate transfection techniques. The transfected cells were cultured at 37°C in 5% CO₂ in DMEM F12 50:50 supplemented with 10% FBS, 100mM HEPES (pH 7.2) and 1mM glutamine. The WSX receptor immunoadhesin was purified using a ProSepATM protein A column.

EXAMPLE 3

Antibody Production

In order to raise antibodies against the WSX receptor, the WSX receptor immunoadhesin of Example 2 was used to inoculate rabbits to raise polyclonal antibodies and mice to raise monoclonal antibodies using conventional technology.

EXAMPLE 4

Generation of a Cell Line Expressing WSX Receptor

The nucleic acid encoding full length WSX receptor variant 13.2 was inserted in the pRKtkNeo plasmid (Holmes *et al.*, *Science* 253:1278-1280 (1991)). 100 µg of the pRKtkNeo.WSX plasmid thus generated was linearized, ethanol precipitated and resuspended in 100 µL of RPMI 1640. 7 x 10⁶ Baf3 cells (5 x 10⁵/ml) were suspended in 900 µL of RPMI and added to the linearized plasmid. Following electroporation at 325V, 1180 µF using a BRL electroporation apparatus, the cells were plated into 15 mls of RPMI 1640 containing 5% WEHI3B conditioned media and 15% serum. 48 hours later cells were selected in 2mg/ml G418.

To obtain the Baf3/WSX cell line expressing WSX receptor variant 13.2, the G418 selected clones were analyzed by FACS using the rabbit polyclonal antisera raised against the WSX-Fc chimeric protein as described above. The highest expressing clone (designated E6) was sorted by FACS to maintain a population with a high level of WSX receptor expression.

EXAMPLE 5**Role of WSX Receptor in Cellular Proliferation**

The proliferative potentials of WSX receptor variants 13.2 and 12.1 were tested by constructing human growth hormone receptor-WSX receptor (GH-WSX) fusions encoding chimeric proteins consisting of the GH receptor extracellular and transmembrane domains and the WSX receptor variant 13.2 or 12.1 intracellular domains. These chimeric gene fusions were transfected into the IL-3 dependent cell line Baf3. The ability of the GH-WSX transfected Baf3 cells to respond to exogenous growth hormone (GH) was tested in a thymidine incorporation assay. As can be seen in Figs. 6 and 8, the GH-WSX receptor variant 13.2 chimera was capable of increasing thymidine uptake in the transfected Baf3 cells, thus indicating the proliferative potential of the WSX receptor variant 13.2. However, WSX receptor variant 12.1 was unable to transmit a proliferative signal in this experiment (Fig. 8).

Materials and Methods

Recombinant PCR was used to generate the chimeric receptors containing the extracellular and transmembrane domains of the hGH receptor and the cytoplasmic domain of either WSX receptor variant 12.1 or variant 13.2. In short, the cytoplasmic domain of either variant 12.1 or 13.2 beginning with Arg at amino acid 866 and extending down to amino acid 958 or amino acid 1165 respectively, was fused in frame, by sequential PCR, to the hGH receptor extracellular and transmembrane domain beginning with Met at amino acid 18 and extending down to Arg at amino acid 274. The GH-WSX chimera was constructed by first using PCR to generate the extracellular and transmembrane domain of the human GH receptor. The 3' end primer used for this PCR contained 20 nucleotides at the 5' end of the primer corresponding to the first 20 nucleotides of the WSX cytoplasmic domain. The 3' end of the chimera was generated using PCR where the 5' end primer contained the last 19 nucleotides of the human GH receptor transmembrane domain. To generate the full length chimera, the 5' end of the human GH receptor product was combined with the 3' end WSX receptor cytoplasmic PCR product and subsequently amplified to create a fusion of the two products.

This chimeric fusion was digested with ClaI and XbaI and ligated to pRKtkNeo (Holmes *et al.*, *Science* 253:1278-1280 (1991)) to create the chimeric expression vector. The IL-3 dependent cell line Baf3 was then electroporated with this hGH/WSX chimeric expression vector.

Briefly, 100µg of the pRKtkNeo/GH.WSX plasmid was linearized, ethanol precipitated and resuspended in 100 µL of RPMI 1640. 7×10^6 Baf3 cells (5×10^5 /ml) were suspended in 900 µL of RPMI and added to the linearized plasmid. Following electroporation at 325V, 1180 µF using a BRL electroporation apparatus, the cells were plated into 15 mls of RPMI 1640 containing 5% wehi conditioned media and 15% serum. 48 hours later, cells were selected in 2mg/ml G418.

To obtain the Baf3/GH.WSX cell lines, the G418 selected cells were FACS sorted using an anti-human GH mAb (3B7) at 1µg/ml. The top 10% expressing cells were selected and expanded.

EXAMPLE 6**Expression Analysis of the WSX Receptor**

The expression profile of the WSX receptor was initially examined by Northern analysis. Northern blots of human fetal or adult tissue mRNA were obtained from Clontech (Palo Alto, California). A transcript of approximately 6 kb was detected in human fetal lung, liver and kidney. In the adult, low level expression was

detected in a variety of tissues including liver, placenta, lung skeletal muscle, kidney, ovary, prostate and small intestine.

PCR analysis of human cord blood identified transcripts in CD34⁺ subfraction. By PCR analysis, all three variants of the WSX receptor were present in CD34⁺ cells. The CD34⁺ subfraction appeared negative by this same PCR analysis.

By PCR analysis, both the 6.4 variant and 13.2 variant were evident in the AA4⁺Sca⁺Kit⁺ (f1ASK) cell population isolated from the mid-gestation fetal liver as described in Zeigler *et al.*, *Blood* 84:2422-2430 (1994). No clones containing the 12.1 variant cytoplasmic tail have been isolated from murine tissues.

Human B cells isolated from peripheral blood using anti-CD19/20 antibodies were also positive for short form (6.4 variant) and long form (13.2 variant) receptor mRNA expression.

The WSX receptor appears to be expressed on both progenitor and more mature hematopoietic cells.

EXAMPLE 7

Cloning of Murine WSX Receptor

The human WSX receptor was used as a probe to isolate murine WSX receptor. The pRtkNeo.WSX plasmid of Example 4 was digested using SspI. This SspI fragment (1624 bps) was isolated, and radiolabelled, and used to screen a murine liver λ gt10 library (Clontech). This resulted in 4 positive clones which were isolated and sequenced after sub-cloning into pBSSK⁺ via EcoRI digestion. The resultant clones, designated 1, 2, 3, 4 showed homology to the extracellular domain of the human WSX receptor; the contiguous sequences resulting from these clones extended from the initiation methionine to tryptophan at position 783. The overall similarity of human WSX receptor and murine WSX receptor is 73 % over this region of the respective extracellular domains (see Figs. 4A-B).

EXAMPLE 8

The Role of WSX Receptor in Hematopoietic Cell Proliferation

The presence of the WSX receptor in the enriched human stem cell population CD34⁺ from cord blood is indicative of a potential role for this receptor in stem cell/progenitor cell proliferation. The proliferation of CD34⁺ human blood cells in methylcellulose media (Stem Cell Technologies) was determined in the presence or absence of WSX receptor antisense oligonucleotides. These experiments were also repeated in the murine hematopoietic system using AA4⁺Sca⁺Kit⁺ stem cells from the murine fetal liver. In both instances, the antisense oligonucleotides statistically significantly inhibited colony formation from the hematopoietic progenitor cells. See Table 1 below. The anti-proliferative effects were most pronounced using the -20 antisense and the +85 antisense oligonucleotide constructs. This inhibition was not lineage specific to any particular myeloid lineage that resulted from the progenitor expansion. The principal effect of the antisense oligonucleotides was a reduction of overall colony numbers. The size of the individual colonies was also reduced.

Antisense oligonucleotide experiments using both human and murine stem cells demonstrated an inhibition of myeloid colony formation. Although, the reduction in myelopoiesis observed in these assays could be prevented by the additional inclusion of G-CSF and GM-CSF in the culture medium. These data serve to illustrate the redundancy of cytokine action in the myelopoietic compartment.

TABLE 1

EXPERIMENT	OLIGO	AVG. COLONY #	% INHIBITION
Human Cord Blood (KL)	(-20)AS	32	
	(-20)S	100	70
	(-20)SCR	114	
	(+85)AS	80	
	(+85)S	123	38
	(+85)SCR	138	
	Control	158	
Human Cord Blood (IL-3, IL-6, KL)	(-20)AS	78	
	(-20)S	188	54
	(-20)SCR	151	
	(+85)AS	167	
	(+85)S	195	18
	(+85)SCR	213	
	Control	266	
Human Cord Blood (KL)	(-20)AS	42	
	(-20)S	146	69
	(-20)SCR	121	
	(+85)AS	123	
	(+85)S	162	23
	(+85)SCR	156	
	Control	145	
Murine Fetal Liver (KL)	(+84)AS	33	
	(+84)S	86	54
	(+84)SCR	57	
	(-20)AS	27	
	(-20)S	126	71
	(-20)SCR	60	
	(-99)AS	109	
	(-99)S	93	0
	(-99)SCR	109	
	Control	121	
Murine Fetal Liver (KL)	(-213)AS	51	
	(-213)S	60	10
	(-213)SCR	53	
	(+211)AS	58	
	(+211)S	54	3
	(+211)SCR	66	
	Control	59	

Materials and Methods

10 *Human stem cells:* Human umbilical cord blood was collected in PBS/Heparin (1000 μ /ml). The nonnuclear fraction was separated using a dextran gradient and any remaining red blood cells lysed in 20 mM NH_4Cl . CD34^+ cells were isolated using CD34^+ immunomagnetic beads (Miltenyi, CA). These isolated CD34^+ cells were found to be 90-97% CD34^+ by FACS analysis.

Murine stem cells: Midgestation fetal liver were harvested and positively selected for the AA4⁺ antigen by immune panning. The AA4⁺ positive fraction was then further enriched for stem cell content by FACS isolation of the AA4⁺ Sca⁺ Kit⁺ fraction.

Antisense experiments: Oligodeoxynucleotides were synthesized against regions of the human or murine WSX receptors. For each oligonucleotide chosen, antisense (AS), sense (S) and scrambled (SCR) versions were synthesized (see Fig. 7). + or - indicates position relative the initiation methionine of the WSX receptor. CD34⁺ or AA4⁺ Sca⁺ Kit⁺ cells were incubated at a concentration of 10³/ml in 50:50 DMEM/F12 media supplemented with 10% FBS, L-glutamine, and GIBCO™ lipid concentrate containing either sense, antisense or scrambled oligonucleotides at a concentration of 70 µg/ml. After 16 hours, a second aliquot of the respective oligonucleotide was added (35 µg/ml) and the cells incubated for a further 6 hours.

Colony assays: 5000 cells from each of the above conditions were aliquoted into 5 ml of methylcellulose (Stem Cell Technologies) containing kit ligand (KL) (25 ng/ml), interleukin-3 (IL-3) (25 ng/ml) and interleukin-6 (IL-6) (50 ng/ml). The methylcellulose cultures were then incubated at 37°C for 14 days and the resultant colonies counted and phenotyped. All assays were performed in triplicate.

EXAMPLE 9

WSX Receptor Variant 13.2 is a Receptor for OB Protein

The WSX receptor variant 13.2 has essentially the same amino acid sequence as the recently cloned leptin (OB) receptor. See Tartaglia *et al.*, *Cell* 83:1263-1271 (1995). OB protein was able to stimulate thymidine incorporation in Baf3 cells transfected with WSX receptor variant 13.2 as described in Example 4 (See Fig. 9).

OB protein expression in hematopoietic cells was studied. Oligonucleotide primers designed specifically against the OB protein illustrated the presence of this ligand in fetal liver and fetal brain as well as in two fetal liver stromal cell lines, designated 10-6 and 7-4. Both of these immortalized stromal cell lines have been demonstrated to support both myeloid and lymphoid proliferation of stem cell populations (Zeigler *et al.*, *Blood* 84:2422-2430 (1994)).

EXAMPLE 10

Role of OB Protein in Hematopoiesis

To examine the hematopoietic activity of OB protein, a variety of *in vitro* assays were performed.

Murine fetal liver fLASK stem cells were isolated from the midgestational fetal liver as described in Zeigler *et al.*, *Blood* 84:2422-2430 (1994) and studied in stem cell suspension culture or methylcellulose assays.

For the stem cell suspension cultures, twenty thousand of the fLASK cells were seeded in individual wells in a 12 well format in DMEM 4.5/F12 media supplemented with 10% heat inactivated fetal calf serum (Hyclone, Logan, UT) and L-glutamine. Growth factors were added at the following concentrations: kit ligand (KL) at 25 ng/mL, interleukin-3 (IL-3) at 25 ng/mL, interleukin-6 (IL-6) at 50 ng/mL, G-CSF at 100 ng/mL, GM-CSF at 100 ng/mL, EPO at 2U/mL, interleukin-7 (IL-7) at 100 ng/mL (all growth factors from R and D Systems,

Minneapolis, MN). OB protein was added at 100 ng/mL unless indicated otherwise. Recombinant OB protein was produced as described in Levin *et al.*, *Proc. Natl. Acad. Sci. (USA)* 93:1726-1730 (1996).

In keeping with its ability to transduce a proliferative signal in Baf3 cells (see previous Example), OB protein dramatically stimulated the expansion of fASK cells grown in suspension culture in the presence of kit ligand (Fig. 10A). The addition of OB protein alone to these suspension cultures was unable to effect survival of the hematopoietic stem cells (HSCs). When a variety of hematopoietic growth factors in suspension culture assays were tested, the main synergy of OB protein appeared to be with KL, GM-CSF and IL-3 (Table 2). No preferential expansion of any particular lineage was observed from cytopsin analysis of the resultant cultures.

TABLE 2

Factor	KL	KL+OB protein	OB protein
N/A	128+/-9	192+/-13	
G-CSF	131+/-3	177+/-8	30+/-5
GM-CSF	148+/-4	165+/-6	134+/-10
IL-3	189+/-7	187+/-4	144+/-
IL-6	112+/-4	198+/-5	32+/-3
EPO	121+/-3	177+/-8	30+/-6
IL-3 & IL-6	112+/-12	198+/-7	32+/-7

fASK stem cells were isolated. Twenty thousand cells were plated in suspension culture with the relevant growth factor combination. Cells were harvested and counted after 7 days. Cell numbers are presented $\times 10^3$. Assays were performed in triplicate and repeated in two independent experiments.

Methylcellulose assays were performed as previously described (Zeiger *et al.*, *supra*). Briefly, methylcellulose colony assays were performed using "complete" methylcellulose or pre-B methylcellulose medium (Stem Cell Technologies, Vancouver, British Columbia, Canada) with the addition of 25 ng/mL KL (R and D Systems, Minneapolis, MN). Cytopsin analyses of the resultant colonies were performed as previously described in Zeigler *et al.*

When these methylcellulose assays were employed, OB protein augmented myeloid colony formation and dramatically increased lymphoid and erythroid colony formation (Figs. 10B and 10C) which demonstrates that OB protein can act on very early cells of the hematopoietic lineage. Importantly, the hematopoietic activity of OB protein was not confined to fetal liver stem cells, the murine bone marrow stem cell population: Lin^{lo}Sca⁺ also proliferated in response to OB protein (KL: 5 fold expansion, KL and OB protein: 10 fold expansion).

Further hematopoietic analysis of the role of the WSX receptor was carried out by examining hematopoietic defects in the *db/db* mouse.

These defects were assessed by measuring the proliferative potential of *db/db* homozygous mutant marrow. Under conditions favoring either myeloid (Humphries *et al.*, *Proc. Natl. Acad. Sci. (USA)* 78:3629-3633 (1981)) or lymphoid (McNiece *et al.*, *J. Immunol.* 146:3785-90 (1991)) expansion, the colony forming potential of the *db/db* marrow was significantly reduced when compared to the wild-type control marrow (Fig. 11). This was particularly evident when the comparison was made under pre-B methylcellulose conditions where KL and IL-7 are used to drive lymphopoiesis (McNiece *et al.*, *supra*). Corresponding analysis of the complementary mouse mutation *ob/ob*, which is deficient in the production of OB protein (Zhang *et al.*, *Nature* 372:425-431 (1994)), also indicated that the lymphoproliferative capacity is compromised in the absence of a functional OB protein signalling pathway (Fig. 11). However, this reduction was less than the reduction observed using *db/db* marrow.

Analysis of the cellular profile of the *db/db* and wild-type marrow revealed significant differences between the two. Overall cellularity of the *db/db* marrow was unchanged. However, when various B cell populations in the *db/db* marrow were examined, both decreased levels of B220⁺ and B220⁺/CD43⁺ cells were found. B220⁺ cells represent all B cell lineages while CD43 is considered to be expressed preferentially on the earliest cells of the B cell hierarchy (Hardy *et al.*, *J. Exp. Med.* 173:1213-25 (1991)). No differences were observed between the CD4/CD8 staining profiles of the two groups. The TER119 (a red cell lineage marker) population was increased in the *db/db* marrow (Fig. 12A).

Comparison of the spleens from the two groups revealed a significant decrease in both tissue weight and cellularity of the *db/db* mice compared to the homozygote misty gray controls (0.063 ± 0.009 g vs. 0.037 ± 0.006 g and $1.10 \times 10^7 \pm 1 \times 10^4$ vs. $4.3 \times 10^6 \pm 10^3$ cells > p0.05). This decreased cellularity in the *db* spleen was reflected in a marked reduction in TER119 staining (Fig. 12B). This result appears to confirm the synergy demonstrated between OB protein and EPO and points to a role for OB protein in the regulation of erythropoiesis.

Examination of the hematopoietic compartment of the *db/db* mouse *in vivo* demonstrated a significant reduction in peripheral blood lymphocytes when compared to heterozygote or wild-type controls. *Db/db* mice fail to regulate blood glucose levels and become diabetic at approximately 6-8 weeks of age; therefore, peripheral blood counts as the animals matured were followed.

For procurement of blood samples, prior to the experiment and at time points throughout the study, 40 µL of blood was taken from the orbital sinus and immediately diluted into 10 mL of diluent to prevent clotting. The complete blood count from each blood sample was measured on a Serrono Baker system 9018 blood analyzer within 60 min. of collection. Only half the animals in each dose group were bled on any given day, thus, each animal was bled on alternate time points. Blood glucose levels were measured in orbital sinus blood samples using One Touch glucose meters and test strips (Johnson and Johnson). The results of this experiment are shown in Figs. 13A-C.

This analysis demonstrated that peripheral blood lymphocytes are significantly reduced at all time points compared to control animals and that the peripheral lymphocyte population of the *db/db* mouse does not change significantly with age. FACS analysis revealed that the decreased lymphocyte population represented a decrease in both B220⁺ cells and CD4/CD8 cells. Both erythrocyte and platelets are at wild-type levels throughout all time periods examined. The peripheral blood lymphocyte levels in *ob/ob* homozygous mutant mice were unchanged from wild-type controls.

Hematopoietic analysis of the *db/db* mouse can be complicated by the onset of diabetes. Therefore, the impact of high glucose levels on lymphopoiesis was examined by comparing the peripheral blood profiles and blood glucose levels in two other diabetic models, the glucokinase knockout heterozygote mouse (Grupe *et al.*, *Cell* 83:69-78 (1995)) and the IFN- α transgenic mouse (Stewart *et al.*, *Science* 260:1942-6 (1993)). Comparison of peripheral lymphocytes and blood glucose in *db/db* mice, their appropriate controls and the high glucose models illustrated no relationship between blood-glucose and lymphocyte counts (Fig. 14). These results suggest therefore that the lymphoid defects observed in the *db/db* mouse are directly attributed to the hematopoietic function of the OB protein signalling pathway.

To test the capacity of the *db/db* hematopoietic compartment to respond to challenge, the *db/db* mice and controls were subjected to sub-lethal irradiation C57BLKS/J *db/db*, C57BLKS/Jm⁺/db, and C57BLKS/Jm⁺/m⁺m mice were subjected to sub-lethal whole body irradiation (750 cGy, 190 cGy/min) as a single dose from a ¹³⁷Cs source. Ten animals were used per experimental group. The kinetics of hematopoietic recovery were then followed by monitoring the peripheral blood during the recovery phase. This experiment illustrated the inability of the *db/db* hematopoietic system to fully recover the lymphopoietic compartment of the peripheral blood 35 days post-irradiation. Platelet levels in these mice followed the same recovery kinetics as controls, however the reduction in erythrocytes lagged behind controls by 7-10 days. This finding may reflect the increased TER 119 population found in the marrow of the *db/db* mice (Fig. 12A).

Materials and Methods

Bone marrow, spleens and peripheral blood was harvested from the diabetic mouse strains: C57BLKS/J *db/db* (mutant), C57BLKS/J m⁺/db (lean heterozygote control littermate), C57BLKS/J+m⁺/m (lean homozygote misty gray coat control littermate) and the obese mouse strains: C57BL/6J-*ob/ob* (mutant) and the C57BL/6J-*ob/+* (lean littermate control). All strains from the Jackson Laboratory, Bar Harbor, ME. A minimum of five animals were used per experimental group. Femurs were flushed with Hank's balanced salt solution (HBSS) plus 2% FCS and a single cell suspension was made of the bone marrow cells. Spleens were harvested and the splenic capsule was ruptured and filtered through a nylon mesh. Peripheral blood was collected through the retro-orbital sinus in phosphate buffered saline (PBS) with 10U/mL heparin and 1mmol EDTA and processed as previously described. The bone marrow, splenocytes and peripheral blood were then stained with the monoclonal antibodies against the following antigens: B220/CD45R (Pan B cell) FITC antimouse, TER-119/erythroid cell R-PE antimouse, CD4 (L3T4), FITC antimouse, CD8 (Ly 3.2), FITC antimouse, and sIgM (Igh-6b), FITC antimouse

(All monoclonals from Pharmigen, San Diego, CA). The appropriate isotype controls were included in each experiment. For methylcellulose assays, the bone marrow from five animals per group was pooled and 100,000 cell aliquots from each group used for each assay point.

EXAMPLE 11

Expression of OB-immunoadhesin

Using protein engineering techniques, the human OB protein was expressed as a fusion with the hinge, CH2 and CH3 domains of IgG1. DNA constructs encoding the chimera of the human OB protein and IgG1 Fc domains were made with the Fc region clones of human IgG1. Human OB cDNA was obtained by PCR from human fat cell cDNA (Clontech Buick-Clone cDNA product). The source of the IgG1 cDNA was the plasmid pBSSK-CH2CH3. The chimera contained the coding sequence of the full length OB protein (amino acids 1-167 in Figure 16) and human IgG1 sequences beginning at aspartic acid 216 (taking amino acid 114 as the first residue of the heavy chain constant region (Kabat *et al.*, *Sequences of Proteins of Immunological Interest* 4th ed. (1987)), which is the first residue of the IgG1 hinge after the cysteine residue involved in heavy-light chain bonding, and ending with residues 441 to include the CH2 and CH3 Fc domains of IgG1. There was an insert of codons for three amino acids (GlyValThr) between the OB protein and IgG1 coding sequences. If necessary, this short linker sequence can easily be deleted, for example by site directed deletion mutagenesis, to create an exact junction between the coding sequences of the OB protein and the IgG1 hinge region. The coding sequence of the OB-IgG1 immunoadhesin was subcloned into the pRK5-based vector pRK5tk-neo which contains a neomycine selectable marker, for transient expression in 293 cells using the calcium phosphate technique (Suva *et al.*, *Science* 237:893-896 (1987)). 293 cells were cultured in HAM's : Low Glucose DMEM medium (50:50), containing 10% FBS and 2 mM L-Gln. For purification of OB-IgG1 chimeras, cells were changed to serum free production medium PS24 the day after transfection and media collected after three days. The culture media was filtered.

The filtered 293 cell supernatant (400 ml) containing recombinant human OB-IgG1 was made 1 mM in phenylmethylsulfonyl fluoride and 2 µg/ml in aprotinin. This material was loaded at 4°C onto a 1 x 4.5 cm Protein A agarose column (Pierce catalog # 20365) equilibrated in 100 mM HEPES pH 8. The flow rate was 75 ml/h. Once the sample was loaded, the column was washed with equilibration buffer until the A₂₈₀ reached baseline. The OB-IgG1 protein was eluted with 3.5 M MgCl₂ + 2% glycerol (unbuffered) at a flow rate of 15 ml/h. The eluate was collected with occasional mixing into 10 ml of 100 mM HEPES pH 8 to reduce the MgCl₂ concentration by approximately one-half and to raise the pH. The eluted protein was then dialyzed into phosphate buffered saline, concentrated, sterile filtered and stored either at 4°C or frozen at -70 °C. The OB-IgG1 immunoadhesin prepared by this method is estimated by SDS-PAGE to be greater than 90% pure.

EXAMPLE 12**Preparation of PEG-OB**

The PEG derivatives of the human OB protein were prepared by reaction of hOB protein purified by reverse phase chromatography with a succinimidyl derivative of PEG propionic acid (SPA-PEG) having a nominal molecular weight of 10 kD, which had been obtained from Shearwater Polymers, Inc. (Huntsville, AL). After purification of the hOB protein by reverse phase chromatography, an approximately 1-2 mg/ml solution of the protein in 0.1% trifluoroacetic acid and approximately 40% acetonitrile, was diluted with 1/3 to 1/2 volume of 0.2 M borate buffer and the pH adjusted to 8.5 with NaOH. SPA-PEG was added to the reaction mixture to make 1:1 and 1:2 molar ratios of protein to SPA-PEG and the mixture was allowed to incubate at room temperature for one hour. After reaction and purification by gel electrophoresis or ion exchange chromatography, the samples were extensively dialyzed against phosphate-buffered saline and sterilized by filtration through a 0.22 micron filter. Samples were stored at 4°C. Under these conditions, the PEG-hOB resulting from the 1:1 molar ratio protein to SPA-PEG reaction consisted primarily of molecules with one 10 kD PEG attached with minor amounts of the 2 PEG-containing species. The PEG-hOB from the 1:2 molar reaction consisted of approximately equal amounts of 2 and 3 PEGs attached to hOB, as determined by SDS gel electrophoresis. In both reactions, small amounts of unreacted protein were also detected. This unreacted protein can be efficiently removed by the gel filtration or ion exchange steps as needed. The PEG derivatives of the human OB protein can also be prepared essentially following the aldehyde chemistry described in EP 372,752 published June 13, 1990.

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EXAMPLE 13**Murine Agonist Antibodies**

Mice were immunized five times with 20µg of the WSX receptor immunoaderhin (see Example 2 above) resuspended in MPL-TDM (monophosphoryl lipid A/trehalose dicorynomycolate; Rabi, Immunochemical Research Inc.) into each foot pad. Three days after the last immunization, popliteal lymphoid cells were fused with mouse myeloma cells, X63-Ag8.8.653 cells, using 50% polyethylene glycol as described (Laskov *et al. Cell. Immunol.* 55:251 (1980)).

The initial screening of hybridoma culture supernatants was done using a capture ELISA. For the capture ELISA, microtiter plates (Maxisorb; Nunc, Kamstrup, Denmark) were coated with 50µl/well of 2µg/ml of goat antibodies specific to the Fc portion of human IgG (Goat anti-hIgG-Fc; Cappel), in PBS, overnight at 4°C and blocked with 2x BSA for 1 hr at room temperature. Then, 50µl/well of 2µg/ml of WSX receptor immunoaderhin was added to each well for 1 hr. The remaining anti-Fc binding sites were blocked with PBS containing 3% human serum and 10µg/ml of CD4-IgG for 1 hr. Plates were incubated with 50µl/well of 2µg/ml of anti-WSX receptor monoclonal antibody (or hybridoma culture supernatant) for 1 hr. Plates were then incubated with 50µl/well of HRP-g at anti-mouse IgG. The bound enzyme was detected by the addition of the

substrate (OPD) and the plates were read at 490nm with an ELISA plate reader. Between each step, plates were washed in wash buffer (PBS containing 0.05% TWEEN 20™).

Agonist antibodies were screened for using the KIRA ELISA described in WO95/14930. A chimeric receptor comprising the extracellular domain of the WSX receptor and the transmembrane and intracellular domain of Rse receptor (Mark *et al.*, *Journal of Biological Chemistry* 269(14):10720-10728 (1994)) with a carboxyl-terminal herpes simplex virus glycoprotein D (gD) tag was produced and dp12.CHO cells were transformed therewith as described in Example 4 of WO95/14930.

The WSX/Rse.gD transformed dp12.CHO cells were seeded (3×10^4 per well) in the wells of a flat-bottom-96 well culture plate in 100µl media and cultured overnight at 37°C in 5% CO₂. The following morning the well supernatants were removed and various concentrations of purified mAb were then added to separate wells. The cells were stimulated at 37°C for 30 min. and the well supernatants were decanted. To lyse the cells and solubilize the chimeric receptors, 100 µl of lysis buffer was added to each well. The plate was then agitated gently on a plate shaker (Bellco Instruments, Vineland, NJ) for 60 min. at room temperature.

While the cells were being solubilized, an ELISA microtiter plate (Nunc Maxisorp, Inter Med, Denmark) coated overnight at 4°C with the 5B6 monoclonal anti-gD antibody (5.0 µg/ml in 50 mM carbonate buffer, pH 9.6, 100 µl/well) was decanted and blocked with 150 µl/well of Block Buffer containing 2% BSA for 60 min. at room temperature. After 60 minutes, the anti-gD 5B6 coated plate was washed 6 times with wash buffer (PBS containing 0.05 % TWEEN 20™ and 0.01 % thimerosal).

The lysate containing solubilized WSX/Rse.gD from the cell-culture microtiter well was transferred (85µl/well) to anti-gD 5B6 coated and blocked ELISA well and was incubated for 2 h at room temperature. The unbound WSX/Rse.gD was removed by washing with wash buffer and 100 µl of biotinylated 4G10 (anti-phosphotyrosine) diluted 1:18000 in dilution buffer (PBS containing 0.5 % BSA, 0.05 % Tween-20, 5 mM EDTA, and 0.01 % thimerosal), *i.e.* 56 ng/ml was added to each well. After incubation for 2 h at room temperature the plate was washed and HRPO-conjugated streptavidin (Zymed Laboratories, S. San Francisco, CA) was added to each well. The plate was incubated for 30 minutes at room temperature with gentle agitation. The free avidin-conjugate was washed away and 100 µl freshly prepared substrate solution (tetramethyl benzidine (TMB); 2-component substrate kit; Kirkegaard and Perry, Gaithersburg, MD) was added to each well. The reaction was allowed to proceed for 10 minutes, after which the color development was stopped by the addition of 100µl/well 1.0 M H₃PO₄. The absorbance at 450 nm was read with a reference wavelength of 650 nm (ABS_{450/650}), using a *vmax* plate reader (Molecular Devices, Palo Alto, CA) controlled with a Macintosh Centris 650 (Apple Computers, Cupertino, CA) and DeltaSoft software (BioMetallics, Inc, Princeton, NJ).

Four of the 25 anti-WSX receptor monoclonal antibodies activated the chimeric WSX/Rse receptor in the KIRA ELISA. The antibodies were designated: 2D7, 1G4, 1E11 and 1C11.

To determine whether the four agonist anti-WSX receptor mAbs recognized the same or different epitopes, a competitive binding ELISA was performed as described in Kim *et al. J. Immunol. Method* 156:9-17

(1992) using biotinylated mAbs (Bio-mAb). Bio-mAb were prepared using N-hydroxyl succinimide as described in *Antibodies, A Laboratory Manual* Cold Spring Harbor Laboratory, Eds. Harlow E. and D. Lane, p. 341 (1988). Microtiter wells were coated with 50µl of Goat anti-hIgG-Fc and kept overnight at 4°C, blocked with 2% BSA for 1 hr, and incubated with 25 µl/well of human WSX receptor immunoadhesin (1µg/ml) for 1 hr at room temperature. After washing, a mixture of a predetermined optimal concentration of Bio-mAb bound and a thousand-fold excess of unlabeled mAb was added into each well. Following 1hr incubation at room temperature, plates were washed and the amount of Bio-mAb was detected by the addition of HRP-streptavidin. After washing the plates, the bound enzyme was detected by the addition of the substrate o-phenylenediamine dihydrochloride (OPD), and the plates were read at 490nm with an ELISA plate reader.

10 The ability of the mAbs to recognize murine WSX receptor was determined in a capture ELISA. Murine WSX receptor (Fig. 21) fused to a gD tag (see above) was captured by an anti-gD (5B6) coated ELISA plate. After washing, various concentrations of biotinylated mAbs were added into each well. Biotinylated mAbs bound to murine WSX receptor-gD were detected using HRP-streptavidin as described above.

To determine whether the antibodies bound membrane-bound receptor, FACS analysis was performed using 293 cells transfected with WSX receptor. 10⁵ WSX receptor-transfected 293 cells were resuspended in 100µl of PBS plus 1% fetal calf serum (FCS) and incubated with 2D7 or 1G4 hybridoma cell supernatant for 30 min on ice. After washing, cells were incubated with 100µl of FITC-goat anti-mouse IgG for 30 min at 4°C. Cells were washed twice and resuspended in 150µl of PBS plus 1% FCS and analyzed by FACscan (Becton Dickinson, Mountain View, CA). The antibodies 2D7 and 1G4 bound to membrane WSX receptor according to the FACS analysis.

The properties of agonist antibodies 2D7 and 1G4 are summarized in the following table.

TABLE 2

mAb	Isotype	epitope ^a	hWSXR ^b	mWSXR ^b	Agonist ^c
2D7	IgG1	A	+++	++	+
1G4	IgG1	B	+++	+	+

^a These mAbs are shown to recognize different epitopes by competitive binding ELISA.

^b These results are determined by ELISA (hWSXR is human WSX receptor and mWSXR is murine WSX receptor).

^c The agonistic activities were determined by KIRA ELISA.

30

EXAMPLE 14**Human Agonist Antibodies**

Single-chain Fv (scFv) fragments binding to the human WSX receptor (hWSXR) were isolated from a large human scFv library (Vaughan *et al.* *Nature Biotechnology* 14:309-314 (1996)) using antigen coated on immunotubes or biotinylated antigen in conjunction with streptavidin-coated magnetic beads (Griffiths *et al.*

EMBO J. 13:3245–3260 (1994); and Vaughan *et al.* (1996)). Briefly, immunotubes coated overnight with 10 µg/ml human WSX receptor immunoadhesin (see Example 2 above) in phosphate buffered saline (PBS) were used for three rounds of panning. The humanized antibody, huMAb4D5–8 (Carter *et al. Proc. Natl. Acad. Sci. USA* 89:4285–4289 (1992)) was used to counter-select for antibodies binding to the Fc of the immunoadhesin.

5 This was done by using 1mg/ml huMAb4D5-8 in solution for the panning steps. In addition, human WSX receptor extracellular domain (cleaved from the WSX receptor immunoadhesin with Genenase (Carter *et al. Proteins: Structure, Function and Genetics* 6:240-248 (1989)) was biotinylated and used for three rounds of panning. Individual phage following two or three rounds of panning were characterized by antigen-binding ELISA (Tables 3 and 4).

10

TABLE 3

Panning with human WSX receptor immunoadhesin-coated immunotubes

Round	Phage ELISA		# clones characterized	# BstNI fingerprints
	hWSXR	Fc		
2	74 / 96	0 / 96	74	11 ^a
15 3	191 / 192	1 / 192	58	8 ^a

^a Total of 11 different clones identified.

TABLE 4

Panning with biotinylated human WSX receptor

Round	Phage ELISA		# clones characterized	# BstNI fingerprints
	hWSXR	Fc		
2	8 / 96	0 / 96	8	4 ^a
20 3	49 / 192	1 / 192	49	4 ^a

^a Total of 7 different clones identified.

Clones binding to human WSX receptor were further characterized by BstNI fingerprinting of a PCR fragment encoding the scFv. A total of 18 clones were identified: 11 from the panning using immunotubes and 7 from the panning using biotinylated antigen (there was no overlap between these groups). The DNA for all 18 clones was sequenced.

Anti-huWSXR clones obtained as described above were analyzed for agonist activity in a KIRA-ELISA assay (see above and Fig. 22) firstly as scFv phage and then as scFv. The scFv phage were PEG-precipitated (Carter *et al., Mutagenesis: A Practical Approach*, McPherson, M. ed. IRL Press, Oxford, UK, Chapter 1, pp 1-25 (1991)) and resuspended in PBS prior to screening. To prepare the scFv, DNA from the clones was transformed into 33D3 cells (a non-suppressor strain for expression of soluble protein). The cells were plated

onto 2YT/2%glucose/50µg per ml of carbenicillin and incubated at 37°C overnight. A 5 ml culture (2YTG: 2YT, 2% glucose, 50µg/ml carbenicillin) was inoculated and grown at 30°C overnight. The next morning, the 5ml culture was diluted into 500ml 2YTG media and grown at 30°C until OD₅₅₀ = 0.3. Then, the media was changed from 2YTG into 2YT/50µg/ml carbenicillin/2mM IPTG and grown at 30°C for 4-5 hrs for scFv production. The culture was harvested and the cell pellet was frozen at -20°C. For purification, the cell pellet was resuspended in 10ml shockate buffer (50mM TrisHCl pH8.5, 20% sucrose, 1mM EDTA) and agitated at 4°C for 1hr. The debris was spun down and supernatant was taken to be purified on Ni NTA Superose (Qiagen) column. MgCl₂ was added to the supernatant to 5mM and loaded onto 0.5ml Ni NTA Superose packed into a disposable column. The column was then washed with 2x5ml wash buffer 1 (50mM sodium phosphate, 300mM NaCl, 25mM imidazole pH 8.0) followed by 2x5ml wash 2 buffer (50mM sodium phosphate, 300mM NaCl, 50mM imidazole pH 8.0). The scFv was then eluted with 2.5ml elution buffer (50mM sodium phosphate, 300mM NaCl, 250mM imidazole, pH8.0). The eluted pool was buffer exchanged into PBS with a NAP5 column (Pharmacia) and stored at 4°C.

Clones #3, #4 and #17 were found to have agonist activity as phage and as scFv (see Figs. 23 and 24). The sequences of these agonist clones are shown in Fig. 25. The activity of the antibodies as F(ab')₂ in the KIRA ELISA was assessed, with clone #4 and clone # 17 showing enhanced activity as F(ab')₂. The ability of the antibodies to bind murine WSX receptor in a capture ELISA (see Example 13) was assessed. Clone #4 and clone # 17 bound murine WSX receptor in this assay.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: GENENTECH, INC.
 - (ii) TITLE OF INVENTION: WSX RECEPTOR AND LIGANDS
 - 5 (iii) NUMBER OF SEQUENCES: 51
 - (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Genentech, Inc.
 - (B) STREET: 460 Point San Bruno Blvd
 - (C) CITY: South San Francisco
 - 10 (D) STATE: California
 - (E) COUNTRY: USA
 - (F) ZIP: 94080
 - (v) COMPUTER READABLE FORM:
 - 15 (A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: WinPatin (Genentech)
 - (vi) CURRENT APPLICATION DATA:
 - 20 (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
 - (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/667197
 - (B) FILING DATE: 06/20/96
 - 25 (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/585005
 - (B) FILING DATE: 01/08/96
 - (viii) ATTORNEY/AGENT INFORMATION:
 - 30 (A) NAME: Lee, Wendy M.
 - (B) REGISTRATION NUMBER: 40,378
 - (C) REFERENCE/DOCKET NUMBER: P0986P2PCT
 - (ix) TELECOMMUNICATION INFORMATION:
 - 35 (A) TELEPHONE: 415/225-1994
 - (B) TELEFAX: 415/952-9881
 - (C) TELEX: 910/371-7168
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
 - 40 (A) LENGTH: 4102 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCTCGA GTCGACGGCG GCGTTAAAG CTCTCGTGGC ATTATCCTTC 50
AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTTAGAGGA 100
TTATGGGTGT ACTTCTCTGA AGTAAGATGA TTTGTCAAAA ATTCTGTGTG 150
5 GTTTTGTTAC ATTGGGAATT TATTTATGTG ATAAGTGCCT TTAAGTTGTC 200
ATATCCAATT ACTCCTTGGA GATTAAAGTT GTCTTGCATG CCACCAAATT 250
CAACCTATGA CTACTTCCTT TTGCCTGCTG GACTCTCAA GAATACTTCA 300
AATTCGAATG GACATTATGA GACAGCTGTT GAACCTAAGT TTAATTCAAG 350
TGGTACTCAC TTTTCTAACT TATCCAAAAC AACTTTCCAC TGTTGCTTTC 400
10 GGAGTGAGCA AGATAGAAAC TGCTCCTTAT GTGCAGACAA CATTGAAGGA 450
AAGACATTTG TTTCAACAGT AAATCTTTA GTTTTCAAC AAATAGATGC 500
AAACTGGAAC ATACAGTGCT GGCTAAAAGG AGACTTAAAA TTATTCATCT 550
GTTATGTGGA GTCATTATTT AAGAATCTAT TCAGGAATTA TAACTATAAG 600
GTCCATCTTT TATATGTTCT GCCTGAAGTG TTAGAAGATT CACCTCTGGT 650
15 TCCCCAAAAA GGCAGTTTTC AGATGGTTCA CTGCAATTGC AGTGTTTCATG 700
AATGTTGTGA ATGTCTTGTG CCTGTGCCAA CAGCCAACT CAACGACACT 750
CTCCTTATGT GTTTGAAAAT CACATCTGGT GGAGTAATTT TCCAGTCACC 800
TCTAATGTCA GTTCAGCCCA TAAATATGGT GAAGCCTGAT CCACCATTAG 850
GTTTGCATAT GGAAATCACA GATGATGGTA ATTTAAAGAT TTCTTGGTCC 900
20 AGCCCACCAT TGGTACCATT TCCACTTCAA TATCAAGTGA AATATTCAGA 950
GAATTCTACA ACAGTTATCA GAGAAGCTGA CAAGATTGTC TCAGCTACAT 1000
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AGGGGCAAGA GACTGGATGG CCCAGGAATC TGGAGTGAAT GGAGTACTCC 1100
TCGTGTCTTT ACCACACAAG ATGTCATATA CTTTCCACCT AAAATTCTGA 1150
25 CAAGTGTTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAAAAC 1200
AAGATTGTTT CCTCAAAAGA GATTGTTTGG TGGATGAATT TAGCTGAGAA 1250

AATTCCTCAA AGCCAGTATG ATGTTGTGAG TGATCATGTT AGCAAAGTTA 1300
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GCAGTGTACT GCTGCAATGA ACATGAATGC CATCATCGCT ATGCTGAATT 1400
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5 TAACTAAAAT GACTTGCAGA TGGTCAACCA GTACAATCCA GTCACTTGCG 1500
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10 ACCAACATGT GTCCTTCCTG ATTCTGTGGT GAAGCCACTG CCTCCATCCA 1750
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25 AAATCTTAAT GAAGATGGTG AAATAAAATG GCTTAGAATC TCTTCATCTG 2500
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TTCAGTCTTT ACCCAATATT TATGGAAGGA GTGGGAAAAC CAAAGATAAT 2600

TAATAGTTTC ACTCAAGATG ATATTGAAAA ACACCAGAGT GATGCAGGTT 2650

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15 AGCATCCCAA CATAATTTCA CCACACCTCA CATTCTCAGA AGGATTGGAT 3300

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20 AAATAATATC AACTTAGGAA CTTCTAGTAA GAAGACTTTT GCATCTTACA 3550

TGCCTCAATT CCAAACCTGT TCTACTCAGA CTCATAAGAT CATGGAAAAC 3600

AAGATGTGTG ACCTAACTGT GTAATTTTAC TGAAGAAACC TTCAGATTG 3650

TGTTATAATG GGTAATATAA AGTGTAATAG ATTATAGTTG TGGGTGGGAG 3700

AGAGAAAAGA AACCAGAGTC AAATTTGAAA ATAATTGTTC CAAATGAATG 3750

25 TTGTCTGTTT GTTCTCTCTT AGTAACATAG ACAAAAAATT TGAGAAAGCC 3800

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 TCTTTTGTGA GATGTAATTG TTTTTCAGA GGGCGTGTG TTTTACCTCA 3950
 AGTTTTTGTG TTGTACCAAC ACACACACAC ACACACATTC TTAACACATG 4000
 TCCTTGTTG TTTTGAGAGT ATATTATGTA TTTATATTTT GTGCTATCAG 4050
 5 ACTGTAGGAT TTGAAGTAGG ACTTTCCTAA ATGTTTAAGA TAAACAGAAT 4100
 TC 4102

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1165 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Met	Ile	Cys	Gln	Lys	Phe	Cys	Val	Val	Leu	Leu	His	Trp	Glu	Phe	
	1				5					10				15		
15	Ile	Tyr	Val	Ile	Thr	Ala	Phe	Asn	Leu	Ser	Tyr	Pro	Ile	Thr	Pro	
					20					25				30		
	Trp	Arg	Phe	Lys	Leu	Ser	Cys	Met	Pro	Pro	Asn	Ser	Thr	Tyr	Asp	
					35					40				45		
20	Tyr	Phe	Leu	Leu	Pro	Ala	Gly	Leu	Ser	Lys	Asn	Thr	Ser	Asn	Ser	
					50					55				60		
	Asn	Gly	His	Tyr	Glu	Thr	Ala	Val	Glu	Pro	Lys	Phe	Asn	Ser	Ser	
					65					70				75		
	Gly	Thr	His	Phe	Ser	Asn	Leu	Ser	Lys	Thr	Thr	Phe	His	Cys	Cys	
					80					85				90		
25	Phe	Arg	Ser	Glu	Gln	Asp	Arg	Asn	Cys	Ser	Leu	Cys	Ala	Asp	Asn	
					95					100				105		
	Ile	Glu	Gly	Lys	Thr	Phe	Val	Ser	Thr	Val	Asn	Ser	Leu	Val	Phe	
					110					115				120		
30	Gln	Gln	Ile	Asp	Ala	Asn	Trp	Asn	Ile	Gln	Cys	Trp	Leu	Lys	Gly	
					125					130				135		
	Asp	Leu	Lys	Leu	Phe	Ile	Cys	Tyr	Val	Glu	Ser	Leu	Phe	Lys	Asn	
					140					145				150		

	Leu Phe Arg Asn Tyr Asn Tyr Lys Val His Leu Leu Tyr Val Leu	155	160	165
	Pro Glu Val Leu Glu Asp Ser Pro Leu Val Pro Gln Lys Gly Ser	170	175	180
5	Phe Gln Met Val His Cys Asn Cys Ser Val His Glu Cys Cys Glu	185	190	195
	Cys Leu Val Pro Val Pro Thr Ala Lys Leu Asn Asp Thr Leu Leu	200	205	210
10	Met Cys Leu Lys Ile Thr Ser Gly Gly Val Ile Phe Gln Ser Pro	215	220	225
	Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro	230	235	240
	Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile	245	250	255
15	Ser Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln	260	265	270
	Val Lys Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp	275	280	285
20	Lys Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro	290	295	300
	Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly	305	310	315
	Pro Gly Ile Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr	320	325	330
25	Gln Asp Val Ile Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly	335	340	345
	Ser Asn Val Ser Phe His Cys Ile Tyr Lys Lys Glu Asn Lys Ile	350	355	360
30	Val Pro Ser Lys Glu Ile Val Trp Trp Met Asn Leu Ala Glu Lys	365	370	375
	Ile Pro Gln Ser Gln Tyr Asp Val Val Ser Asp His Val Ser Lys	380	385	390
	Val Thr Phe Phe Asn Leu Asn Glu Thr Lys Pro Arg Gly Lys Phe	395	400	405

	Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His	
	410	415 420
	Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser	
	425	430 435
5	Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser	
	440	445 450
	Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg	
	455	460 465
10	Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His	
	470	475 480
	Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe	
	485	490 495
	Tyr Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr	
	500	505 510
15	Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro	
	515	520 525
	Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro	
	530	535 540
20	Ser Ser Val Lys Ala Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys	
	545	550 555
	Ile Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe	
	560	565 570
	Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Val Gln Trp Lys Met	
	575	580 585
25	Tyr Glu Val Tyr Asp Ala Lys Ser Lys Ser Val Ser Leu Pro Val	
	590	595 600
	Pro Asp Leu Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg	
	605	610 615
30	Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Asn Pro Ala Tyr	
	620	625 630
	Thr Val Val Met Asp Ile Lys Val Pro Met Arg Gly Pro Glu Phe	
	635	640 645
	Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys Glu Lys Asn Val	
	650	655 660

	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Met	Lys	Asn	Asp	Ser	Leu	Cys	Ser	
					665					670					675	
	Val	Gln	Arg	Tyr	Val	Ile	Asn	His	His	Thr	Ser	Cys	Asn	Gly	Thr	
					680					685					690	
5	Trp	Ser	Glu	Asp	Val	Gly	Asn	His	Thr	Lys	Phe	Thr	Phe	Leu	Trp	
					695					700					705	
	Thr	Glu	Gln	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Ile	Asn	Ser	Ile	
					710					715					720	
	Gly	Ala	Ser	Val	Ala	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	
10					725					730					735	
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					740					745					750	
	Ser	Ser	Cys	Val	Ile	Val	Ser	Trp	Ile	Leu	Ser	Pro	Ser	Asp	Tyr	
					755					760					765	
15	Lys	Leu	Met	Tyr	Phe	Ile	Ile	Glu	Trp	Lys	Asn	Leu	Asn	Glu	Asp	
					770					775					780	
	Gly	Glu	Ile	Lys	Trp	Leu	Arg	Ile	Ser	Ser	Ser	Val	Lys	Lys	Tyr	
					785					790					795	
	Tyr	Ile	His	Asp	His	Phe	Ile	Pro	Ile	Glu	Lys	Tyr	Gln	Phe	Ser	
20					800					805					810	
	Leu	Tyr	Pro	Ile	Phe	Met	Glu	Gly	Val	Gly	Lys	Pro	Lys	Ile	Ile	
					815					820					825	
	Asn	Ser	Phe	Thr	Gln	Asp	Asp	Ile	Glu	Lys	His	Gln	Ser	Asp	Ala	
					830					835					840	
25	Gly	Leu	Tyr	Val	Ile	Val	Pro	Val	Ile	Ile	Ser	Ser	Ser	Ile	Leu	
					845					850					855	
	Leu	Leu	Gly	Thr	Leu	Leu	Ile	Ser	His	Gln	Arg	Met	Lys	Lys	Leu	
					860					865					870	
	Phe	Trp	Glu	Asp	Val	Pro	Asn	Pro	Lys	Asn	Cys	Ser	Trp	Ala	Gln	
30					875					880					885	
	Gly	Leu	Asn	Phe	Gln	Lys	Pro	Glu	Thr	Phe	Glu	His	Leu	Phe	Ile	
					890					895					900	
	Lys	His	Thr	Ala	Ser	Val	Thr	Cys	Gly	Pro	Leu	Leu	Leu	Glu	Pro	
					905					910					915	

	Glu Thr Ile Ser Glu Asp Ile Ser Val Asp Thr Ser Trp Lys Asn	
	920	925 930
	Lys Asp Glu Met Met Pro Thr Thr Val Val Ser Leu Leu Ser Thr	
	935	940 945
5	Thr Asp Leu Glu Lys Gly Ser Val Cys Ile Ser Asp Gln Phe Asn	
	950	955 960
	Ser Val Asn Phe Ser Glu Ala Glu Gly Thr Glu Val Thr Tyr Glu	
	965	970 975
10	Asp Glu Ser Gln Arg Gln Pro Phe Val Lys Tyr Ala Thr Leu Ile	
	980	985 990
	Ser Asn Ser Lys Pro Ser Glu Thr Gly Glu Glu Gln Gly Leu Ile	
	995	1000 1005
	Asn Ser Ser Val Thr Lys Cys Phe Ser Ser Lys Asn Ser Pro Leu	
	1010	1015 1020
15	Lys Asp Ser Phe Ser Asn Ser Ser Trp Glu Ile Glu Ala Gln Ala	
	1025	1030 1035
	Phe Phe Ile Leu Ser Asp Gln His Pro Asn Ile Ile Ser Pro His	
	1040	1045 1050
20	Leu Thr Phe Ser Glu Gly Leu Asp Glu Leu Leu Lys Leu Glu Gly	
	1055	1060 1065
	Asn Phe Pro Glu Glu Asn Asn Asp Lys Lys Ser Ile Tyr Tyr Leu	
	1070	1075 1080
	Gly Val Thr Ser Ile Lys Lys Arg Glu Ser Gly Val Leu Leu Thr	
	1085	1090 1095
25	Asp Lys Ser Arg Val Ser Cys Pro Phe Pro Ala Pro Cys Leu Phe	
	1100	1105 1110
	Thr Asp Ile Arg Val Leu Gln Asp Ser Cys Ser His Phe Val Glu	
	1115	1120 1125
30	Asn Asn Ile Asn Leu Gly Thr Ser Ser Lys Lys Thr Phe Ala Ser	
	1130	1135 1140
	Tyr Met Pro Gln Phe Gln Thr Cys Ser Thr Gln Thr His Lys Ile	
	1145	1150 1155
	Met Glu Asn Lys Met Cys Asp Leu Thr Val	
	1160	1165

35 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 896 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

	Met	Ile	Cys	Gln	Lys	Phe	Cys	Val	Val	Leu	Leu	His	Trp	Glu	Phe	
	1				5					10					15	
	Ile	Tyr	Val	Ile	Thr	Ala	Phe	Asn	Leu	Ser	Tyr	Pro	Ile	Thr	Pro	
					20					25					30	
10	Trp	Arg	Phe	Lys	Leu	Ser	Cys	Met	Pro	Pro	Asn	Ser	Thr	Tyr	Asp	
					35					40					45	
	Tyr	Phe	Leu	Leu	Pro	Ala	Gly	Leu	Ser	Lys	Asn	Thr	Ser	Asn	Ser	
					50					55					60	
	Asn	Gly	His	Tyr	Glu	Thr	Ala	Val	Glu	Pro	Lys	Phe	Asn	Ser	Ser	
15					65					70					75	
	Gly	Thr	His	Phe	Ser	Asn	Leu	Ser	Lys	Thr	Thr	Phe	His	Cys	Cys	
					80					85					90	
	Phe	Arg	Ser	Glu	Gln	Asp	Arg	Asn	Cys	Ser	Leu	Cys	Ala	Asp	Asn	
					95					100					105	
20	Ile	Glu	Gly	Lys	Thr	Phe	Val	Ser	Thr	Val	Asn	Ser	Leu	Val	Phe	
					110					115					120	
	Gln	Gln	Ile	Asp	Ala	Asn	Trp	Asn	Ile	Gln	Cys	Trp	Leu	Lys	Gly	
					125					130					135	
	Asp	Leu	Lys	Leu	Phe	Ile	Cys	Tyr	Val	Glu	Ser	Leu	Phe	Lys	Asn	
25					140					145					150	
	Leu	Phe	Arg	Asn	Tyr	Asn	Tyr	Lys	Val	His	Leu	Leu	Tyr	Val	Leu	
					155					160					165	
	Pro	Glu	Val	Leu	Glu	Asp	Ser	Pro	Leu	Val	Pro	Gln	Lys	Gly	Ser	
					170					175					180	
30	Phe	Gln	Met	Val	His	Cys	Asn	Cys	Ser	Val	His	Glu	Cys	Cys	Glu	
					185					190					195	
	Cys	Leu	Val	Pro	Val	Pro	Thr	Ala	Lys	Leu	Asn	Asp	Thr	Leu	Leu	
					200					205					210	
	Met	Cys	Leu	Lys	Ile	Thr	Ser	Gly	Gly	Val	Ile	Phe	Gln	Ser	Pro	
35					215					220					225	

	Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro	
	230	235 240
	Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile	
	245	250 255
5	Ser Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln	
	260	265 270
	Val Lys Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp	
	275	280 285
10	Lys Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro	
	290	295 300
	Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly	
	305	310 315
	Pro Gly Ile Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr	
	320	325 330
15	Gln Asp Val Ile Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly	
	335	340 345
	Ser Asn Val Ser Phe His Cys Ile Tyr Lys Lys Glu Asn Lys Ile	
	350	355 360
20	Val Pro Ser Lys Glu Ile Val Trp Trp Met Asn Leu Ala Glu Lys	
	365	370 375
	Ile Pro Gln Ser Gln Tyr Asp Val Val Ser Asp His Val Ser Lys	
	380	385 390
	Val Thr Phe Phe Asn Leu Asn Glu Thr Lys Pro Arg Gly Lys Phe	
	395	400 405
25	Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His	
	410	415 420
	Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser	
	425	430 435
30	Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser	
	440	445 450
	Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg	
	455	460 465
	Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His	
	470	475 480

	Pro	Ile	Ser	Glu	Pro	Lys	Asp	Cys	Tyr	Leu	Gln	Ser	Asp	Gly	Phe	
					485					490					495	
	Tyr	Glu	Cys	Ile	Phe	Gln	Pro	Ile	Phe	Leu	Leu	Ser	Gly	Tyr	Thr	
					500					505					510	
5	Met	Trp	Ile	Arg	Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	
					515					520					525	
	Pro	Thr	Cys	Val	Leu	Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	
					530					535					540	
10	Ser	Ser	Val	Lys	Ala	Glu	Ile	Thr	Ile	Asn	Ile	Gly	Leu	Leu	Lys	
					545					550					555	
	Ile	Ser	Trp	Glu	Lys	Pro	Val	Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	
					560					565					570	
	Gln	Ile	Arg	Tyr	Gly	Leu	Ser	Gly	Lys	Glu	Val	Gln	Trp	Lys	Met	
					575					580					585	
15	Tyr	Glu	Val	Tyr	Asp	Ala	Lys	Ser	Lys	Ser	Val	Ser	Leu	Pro	Val	
					590					595					600	
	Pro	Asp	Leu	Cys	Ala	Val	Tyr	Ala	Val	Gln	Val	Arg	Cys	Lys	Arg	
					605					610					615	
20	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	Trp	Ser	Asn	Pro	Ala	Tyr	
					620					625					630	
	Thr	Val	Val	Met	Asp	Ile	Lys	Val	Pro	Met	Arg	Gly	Pro	Glu	Phe	
					635					640					645	
	Trp	Arg	Ile	Ile	Asn	Gly	Asp	Thr	Met	Lys	Lys	Glu	Lys	Asn	Val	
					650					655					660	
25	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Met	Lys	Asn	Asp	Ser	Leu	Cys	Ser	
					665					670					675	
	Val	Gln	Arg	Tyr	Val	Ile	Asn	His	His	Thr	Ser	Cys	Asn	Gly	Thr	
					680					685					690	
30	Trp	Ser	Glu	Asp	Val	Gly	Asn	His	Thr	Lys	Phe	Thr	Phe	Leu	Trp	
					695					700					705	
	Thr	Glu	Gln	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Ile	Asn	Ser	Ile	
					710					715					720	
	Gly	Ala	Ser	Val	Ala	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	
					725					730					735	

Ser Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn
 740 745 750
 Ser Ser Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr
 755 760 765
 5 Lys Leu Met Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp
 770 775 780
 Gly Glu Ile Lys Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr
 785 790 795
 Tyr Ile His Asp His Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser
 10 800 805 810
 Leu Tyr Pro Ile Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile
 815 820 825
 Asn Ser Phe Thr Gln Asp Asp Ile Glu Lys His Gln Ser Asp Ala
 830 835 840
 15 Gly Leu Tyr Val Ile Val Pro Val Ile Ile Ser Ser Ser Ile Leu
 845 850 855
 Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu
 860 865 870
 Phe Trp Glu Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln
 20 875 880 885
 Gly Leu Asn Phe Gln Lys Arg Thr Asp Ile Leu
 890 895 896

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 25 (A) LENGTH: 923 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe
 30 1 5 10 15
 Ile Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro
 20 25 30
 Trp Arg Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp
 35 40 45

	Tyr	Phe	Leu	Leu	Pro	Ala	Gly	Leu	Ser	Lys	Asn	Thr	Ser	Asn	Ser
					50					55					60
	Asn	Gly	His	Tyr	Glu	Thr	Ala	Val	Glu	Pro	Lys	Phe	Asn	Ser	Ser
					65					70					75
5	Gly	Thr	His	Phe	Ser	Asn	Leu	Ser	Lys	Thr	Thr	Phe	His	Cys	Cys
					80					85					90
	Phe	Arg	Ser	Glu	Gln	Asp	Arg	Asn	Cys	Ser	Leu	Cys	Ala	Asp	Asn
					95					100					105
10	Ile	Glu	Gly	Lys	Thr	Phe	Val	Ser	Thr	Val	Asn	Ser	Leu	Val	Phe
					110					115					120
	Gln	Gln	Ile	Asp	Ala	Asn	Trp	Asn	Ile	Gln	Cys	Trp	Leu	Lys	Gly
					125					130					135
	Asp	Leu	Lys	Leu	Phe	Ile	Cys	Tyr	Val	Glu	Ser	Leu	Phe	Lys	Asn
					140					145					150
15	Leu	Phe	Arg	Asn	Tyr	Asn	Tyr	Lys	Val	His	Leu	Leu	Tyr	Val	Leu
					155					160					165
	Pro	Glu	Val	Leu	Glu	Asp	Ser	Pro	Leu	Val	Pro	Gln	Lys	Gly	Ser
					170					175					180
20	Phe	Gln	Met	Val	His	Cys	Asn	Cys	Ser	Val	His	Glu	Cys	Cys	Glu
					185					190					195
	Cys	Leu	Val	Pro	Val	Pro	Thr	Ala	Lys	Leu	Asn	Asp	Thr	Leu	Leu
					200					205					210
	Met	Cys	Leu	Lys	Ile	Thr	Ser	Gly	Gly	Val	Ile	Phe	Gln	Ser	Pro
					215					220					225
25	Leu	Met	Ser	Val	Gln	Pro	Ile	Asn	Met	Val	Lys	Pro	Asp	Pro	Pro
					230					235					240
	Leu	Gly	Leu	His	Met	Glu	Ile	Thr	Asp	Asp	Gly	Asn	Leu	Lys	Ile
					245					250					255
30	Ser	Trp	Ser	Ser	Pro	Pro	Leu	Val	Pro	Phe	Pro	Leu	Gln	Tyr	Gln
					260					265					270
	Val	Lys	Tyr	Ser	Glu	Asn	Ser	Thr	Thr	Val	Ile	Arg	Glu	Ala	Asp
					275					280					285
	Lys	Ile	Val	Ser	Ala	Thr	Ser	Leu	Leu	Val	Asp	Ser	Ile	Leu	Pro
					290					295					300

	Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly	
	305	310 315
	Pro Gly Ile Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr	
	320	325 330
5	Gln Asp Val Ile Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly	
	335	340 345
	Ser Asn Val Ser Phe His Cys Ile Tyr Lys Lys Glu Asn Lys Ile	
	350	355 360
10	Val Pro Ser Lys Glu Ile Val Trp Trp Met Asn Leu Ala Glu Lys	
	365	370 375
	Ile Pro Gln Ser Gln Tyr Asp Val Val Ser Asp His Val Ser Lys	
	380	385 390
	Val Thr Phe Phe Asn Leu Asn Glu Thr Lys Pro Arg Gly Lys Phe	
	395	400 405
15	Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His	
	410	415 420
	Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser	
	425	430 435
20	Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser	
	440	445 450
	Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg	
	455	460 465
	Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His	
	470	475 480
25	Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe	
	485	490 495
	Tyr Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr	
	500	505 510
30	Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro	
	515	520 525
	Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro	
	530	535 540
	Ser Ser Val Lys Ala Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys	
	545	550 555

	Ile Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe	560	565	570
	Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Val Gln Trp Lys Met	575	580	585
5	Tyr Glu Val Tyr Asp Ala Lys Ser Lys Ser Val Ser Leu Pro Val	590	595	600
	Pro Asp Leu Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg	605	610	615
10	Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Asn Pro Ala Tyr	620	625	630
	Thr Val Val Met Asp Ile Lys Val Pro Met Arg Gly Pro Glu Phe	635	640	645
	Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys Glu Lys Asn Val	650	655	660
15	Thr Leu Leu Trp Lys Pro Leu Met Lys Asn Asp Ser Leu Cys Ser	665	670	675
	Val Gln Arg Tyr Val Ile Asn His His Thr Ser Cys Asn Gly Thr	680	685	690
20	Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr Phe Leu Trp	695	700	705
	Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn Ser Ile	710	715	720
	Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro Met	725	730	735
25	Ser Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn	740	745	750
	Ser Ser Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr	755	760	765
30	Lys Leu Met Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp	770	775	780
	Gly Glu Ile Lys Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr	785	790	795
	Tyr Ile His Asp His Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser	800	805	810

	Leu Tyr Pro Ile Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile	
	815	820 825
	Asn Ser Phe Thr Gln Asp Asp Ile Glu Lys His Gln Ser Asp Ala	
	830	835 840
5	Gly Leu Tyr Val Ile Val Pro Val Ile Ile Ser Ser Ser Ile Leu	
	845	850 855
	Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu	
	860	865 870
10	Phe Trp Glu Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln	
	875	880 885
	Gly Leu Asn Phe Gln Lys Met Phe Arg Thr Pro Arg Ile Val Pro	
	890	895 900
	Gly His Lys Asp Leu Ile Phe Arg Arg Cys Leu Lys Ala Ala Cys	
	905	910 915
15	Ser Leu Arg Val Ile Thr Thr Pro	
	920	923

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 3004 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCGGGG TTAAAGCTCT CGTGGCATT A TCCTTCAGTG GGGCTATTGG 50

25 ACTGACTTTT CTTATGCTGG GATGTGCCTT AGAGGATTAT GGATTGCCA 100

GTTCAACCCTG ACCATCTTGA AAATAAGTTA TCTCTGATCT CTGTCTGTAT 150

GTTACTTCTC TCCCCTCACC AATGGAGAAC AAATGTGGGC AAAGTGTACT 200

TCTCTGAAGT AAGATGATTT GTCAAAAATT CTGTGTGGTT TTGTTACATT 250

GGGAATTTAT TTATGTGATA ACTGCGTTA ACTTGTCATA TCCAATTACT 300

30 CCTTGGAGAT TTAAGTTGTC TTGCATGCCA CCAAATTCAA CCTATGACTA 350

CTTCCTTTTG CCTGCTGGAC TCTCAAAGAA TACTTCAAAT TCGAATGGAC 400

ATTATGAGAC AGCTGTTGAA CCTAAGTTTA ATTCAAGTGG TACTCACTTT 450

TCTAACTTAT CCAAAACAAC TTTCCACTGT TGCTTTCGGA GTGAGCAAGA 500
 TAGAACTGTC TCCTTATGTG CAGACAACAT TGAAGGAAAG ACATTTGTTT 550
 CNACAGTAAA TTCTTTAGTT TTTCAACAAA TAGATGCAAA CTGGAACATA 600
 CAGTGCTGGC TAAAAGGAGA CTTAAAATTA TTCATCTGTT ATGTGGAGTC 650
 5 ATTATTTAAG AATCTATTCA GGAATTATAA CTATAAGGTC CATCTTTTAT 700
 ATGTTCTGCC TGAAGTGTTA GAAGATTCAC CTCTGGTTCC CCAAAAAGGC 750
 AGTTTTCAGA TGGTTCAC TGCAATTGAGT GTTCATGAAT GTTGTGAATG 800
 TCTTGTGCCT GTGCCAACAG CCAAACTCAA CGACACTCTC CTTATGTGTT 850
 TGAAAATCAC ATCTGGTGGA GTAATTTTCC AGTCACCTCT AATGTCAGTT 900
 10 CAGCCCATAA ATATGGTGAA GCCTGATCCA CCATTAGGTT TGCATATGGA 950
 AATCACAGAT GATGGTAATT TAAAGATTTC TTGGTCCAGC CCACCATG 1000
 TACCATTTCC ACTTCAATAT CAAGTGAAAT ATTCAGAGAA TTCTACAACA 1050
 GTTATCAGAG AAGCTGACAA GATTGTCTCA GCTACATCCC TGCTAGTAGA 1100
 CAGTATACTT CCTGGGTCTT CGTATGAGGT TCAGGTGAGG GGCAAGAGAC 1150
 15 TGGATGGCCC AGGAATCTGG AGTGACTGGA GTACTCCTCG TGTCTTTACC 1200
 ACACAAGATG TCATATACTT TCCACCTAAA ATTCTGACAA GTGTGGGGTC 1250
 TAATGTTTCT TTTCACTGCA TCTATAAGAA GGAAAACAAG ATTGTTCCCT 1300
 CAAAAGAGAT TGTTTGGTGG ATGAATTTAG CTGAGAAAAT TCCTCAAAGC 1350
 CAGTATGATG TTGTGAGTGA TCATGTTAGC AAAGTTACTT TTTTCAATCT 1400
 20 GAATGAAACC AAACCTCGAG GAAAGTTTAC CTATGATGCA GTGTACTGCT 1450
 GCAATGAACA TGAATGCCAT CATCGCTATG CTGAATTATA TGTGATTGAT 1500
 GTCAATATCA ATATCTCATG TGAACTGAT GGGTACTTAA CTAAAATGAC 1550
 TTGCAGATGG TCAACCAGTA CAATCCAGTC ACTTGCGGAA AGCACTTTGC 1600
 AATTGAGGTA TCATAGGAGC AGCCTTTACT GTTCTGATAT TCCATCTATT 1650
 25 CATCCCATAT CTGAGCCCAA AGATTGCTAT TTGCAGAGTG ATGGTTTTTA 1700
 TGAATGCATT TTCCAGCCAA TCTTCCTATT ATCTGGCTAC ACAATGTGGA 1750

TTAGGATCAA TCACTCTCTA GGTTCACCTG ACTCTCCACC AACATGTGTC 1800
CTTCCTGATT CTGTGGTGAA GCCACTGCCT CCATCCAGTG TGAAAGCAGA 1850
AATTACTATA AACATTGGAT TATTGAAAAT ATCTTGGGAA AAGCCAGTCT 1900
TTCCAGAGAA TAACCTTCAA TTCCAGATTC GCTATGGTTT AAGTGGAAAA 1950
5 GAAGTACAAT GGAAGATGTA TGAGGTTTAT GATGCAAAAT CAAAATCTGT 2000
CAGTCTCCCA GTTCCAGACT TGTGTGCAGT CTATGCTGTT CAGGTGCGCT 2050
GTAAGAGGCT AGATGGACTG GGATATTGGA GTAATTGGAG CAATCCAGCC 2100
TACACAGTTG TCATGGATAT AAAAGTTCCT ATGAGAGGAC CTGAATTTTG 2150
GAGAATAATT AATGGAGATA CTATGAAAAA GGAGAAAAAT GTCACTTTAC 2200
10 TTTGGAAGCC CCTGATGAAA AATGACTCAT TGTGCAGTGT TCAGAGATAT 2250
GTGATAAACC ATCATACTTC CTGCAATGGA ACATGGTCAG AAGATGTGGG 2300
AAATCACACG AAATTCACCT TCCTGTGGAC AGAGCAAGCA CATACTGTTA 2350
CGGTTCTGGC CATCAATTCA ATTGGTGCTT CTGTTGCAAA TTTTAATTTA 2400
ACCTTTTCAT GGCCTATGAG CAAAGTAAAT ATCGTGCAGT CACTCAGTGC 2450
15 TTATCCTTTA AACAGCAGTT GTGTGATTGT TTCCTGGATA CTATCACCCA 2500
GTGATTACAA GCTAATGTAT TTTATTATTG AGTGGAAAAA TCTTAATGAA 2550
GATGGTGAAA TAAAATGGCT TAGAATCTCT TCATCTGTTA AGAAGTATTA 2600
TATCCATGAT CATTTTATCC CCATTGAGAA GTACCAGTTC AGTCTTTACC 2650
CAATATTTAT GGAAGGAGTG GGAAAACCAA AGATAATTAA TAGTTTCACT 2700
20 CAAGATGATA TTGAAAAACA CCAGAGTGAT GCAGGTTTAT ATGTAATTGT 2750
GCCAGTAATT ATTCCTCTT CCATCTTATT GCTTGAACA TTATTAATAT 2800
CACACCAAAG AATGAAAAAG CTATTTTGGG AAGATGTTCC GAACCCCAAG 2850
AATTGTTCTT GGGCACAAGG ACTTAATTTT CAGAAGAGAA CGGACATTCT 2900
TTGAAGTCTA ATCATGATCA CTACAGATGA ACCCAATGTG CCAACTTCCC 2950
25 AACAGTCTAT AGAGTATTAG AAGATTTTTA CATTTTGAAG AAGGGCCGGA 3000
ATTC 3004

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3102 base pairs
(B) TYPE: Nucleic Acid
5 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAATTCTCGA GTCGACGGCG GGC GTTAAAG CTCTCGTGGC ATTATCCTTC 50
AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTTAGAGGA 100
10 TTATGGGTGT ACTTCTCTGA AGTAAGATGA TTTGTCAAAA ATTCTGTGTG 150
GTTTTGTAC ATTGGGAATT TATTTATGTG ATAAC TGCCT TTAAC TTGTC 200
ATATCCAATT ACTCCTTGA GATTTAAGTT GTCTTGCATG CCACCAAATT 250
CAACCTATGA CTA CTTCCTT TTGCCTGCTG GACTCTCAA GAATACTTCA 300
AATTCGAATG GACATTATGA GACAGCTGTT GAACCTAAGT TTAATTCAAG 350
15 TGGTACTCAC TTTTCTAACT TATCCAAAAC AACTTTCCAC TGTTGCTTTC 400
GGAGTGAGCA AGATAGAAAC TGCTCCTTAT GTGCAGACAA CATTGAAGGA 450
AAGACATTTG TTTCAACAGT AAATTCTTTA GTTTTTCAAC AAATAGATGC 500
AAACTGGAAC ATACAGTGCT GGCTAAAAGG AGACTTAAAA TTATTCATCT 550
GTTATGTGGA GTCATTATTT AAGAATCTAT TCAGGAATTA TAACTATAAG 600
20 GTCCATCTTT TATATGTTCT GCCTGAAGTG TTAGAAGATT CACCTCTGGT 650
TCCCCAAAAA GGCAGTTTTT AGATGGTTCA CTGCAATTGC AGTGTTTCATG 700
AATGTTGTGA ATGTCTTGTG CCTGTGCCAA CAGCCAACT CAACGACACT 750
CTCCTTATGT GTTTGAAAAT CACATCTGGT GGAGTAATTT TCCAGTCACC 800
TCTAATGTCA GTTCAGCCCA TAAATATGGT GAAGCCTGAT CCACCATTAG 850
25 GTTTGCATAT GGAAATCACA GATGATGGTA ATTTAAAGAT TTCTTGGTCC 900
AGCCCACCAT TGGTACCATT TCCACTTCAA TATCAAGTGA AATATTCAGA 950
GAATTCTACA ACAGTTATCA GAGAAGCTGA CAAGATTGTC TCAGCTACAT 1000
CCCTGCTAGT AGACAGTATA CTTCTGGGT CTTCGTATGA GGTTCAGGTG 1050

AGGGGCAAGA GACTGGATGG CCCAGGAATC TGGAGTGAAT GGAGTACTCC 1100
TCGTGTCTTT ACCACACAAG ATGTCATATA CTTTCCACCT AAAATTCTGA 1150
CAAGTGTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAAAAC 1200
AAGATTGTTC CCTCAAAAGA GATTGTTTGG TGGATGAATT TAGCTGAGAA 1250
5 AATTCCTCAA AGCCAGTATG ATGTTGTGAG TGATCATGTT AGCAAAGTTA 1300
CTTTTTTCAA TCTGAATGAA ACCAAACCTC GAGGAAAGTT TACCTATGAT 1350
GCAGTGTACT GCTGCAATGA ACATGAATGC CATCATCGCT ATGCTGAATT 1400
ATATGTGATT GATGTCAATA TCAATATCTC ATGTGAAACT GATGGGTACT 1450
TAACTAAAAT GACTTGCAGA TGGTCAACCA GTACAATCCA GTCACCTGCG 1500
10 GAAAGCACTT TGCAATTGAG GTATCATAGG AGCAGCCTTT ACTGTTCTGA 1550
TATTCATCT ATTATCCCA TATCTGAGCC CAAAGATTGC TATTTGCAGA 1600
GTGATGGTTT TTATGAATGC ATTTTCCAGC CAATCTTCCT ATTATCTGGC 1650
TACACAATGT GGATTAGGAT CAATCACTCT CTAGGTTTAC TTGACTCTCC 1700
ACCAACATGT GTCCTTCCTG ATTCTGTGGT GAAGCCACTG CCTCCATCCA 1750
15 GTGTGAAAGC AGAAATTACT ATAAACATTG GATTATTGAA AATATCTTGG 1800
GAAAAGCCAG TCTTTCCAGA GAATAACCTT CAATTCCAGA TTCGCTATGG 1850
TTTAAGTGGA AAAGAAGTAC AATGGAAGAT GTATGAGGTT TATGATGCAA 1900
AATCAAAATC TGTCACTCTC CCAGTTCCAG ACTTGTGTGC AGTCTATGCT 1950
GTTTCAGTGC GCTGTAAGAG GCTAGATGGA CTGGGATATT GGAGTAATTG 2000
20 GAGCAATCCA GCCTACACAG TTGTCATGGA TATAAAAGTT CCTATGAGAG 2050
GACCTGAATT TTGGAGAATA ATTAATGGAG ATACTATGAA AAAGGAGAAA 2100
AATGTCACTT TACTTTGGAA GCCCCTGATG AAAAATGACT CATTGTGCAG 2150
TGTTTCAGAGA TATGTGATAA ACCATCATAC TTCCTGCAAT GGAACATGGT 2200
CAGAAGATGT GGGAAATCAC ACGAAATTCA CTTTCCTGTG GACAGAGCAA 2250
25 GCACATACTG TTACGGTTCT GGCCATCAAT TCAATTGGTG CTTCTGTTGC 2300
AAATTTTAAT TTAACCTTTT CATGGCCTAT GAGCAAAGTA AATATCGTGC 2350

AGTCACTCAG TGCTTATCCT TTAAACAGCA GTTGTGTGAT TGTTTCCTGG 2400
 ATACTATCAC CCAGTGATTA CAAGCTAATG TATTTTATTA TTGAGTGGAA 2450
 AAATCTTAAT GAAGATGGTG AAATAAAATG GCTTAGAATC TCTTCATCTG 2500
 TTAAGAAGTA TTATATCCAT GATCATTTTA TCCCCATTGA GAAGTACCAG 2550
 5 TTCAGTCTTT ACCCAATATT TATGGAAGGA GTGGGAAAAC CAAAGATAAT 2600
 TAATAGTTTC ACTCAAGATG ATATTGAAAA ACACCAGAGT GATGCAGGTT 2650
 TATATGTAAT TGTGCCAGTA ATTATTTCTT CTTCATCTT ATTGCTTGGA 2700
 ACATTATTAA TATCACACCA AAGAATGAAA AAGCTATTTT GGGAAGATGT 2750
 TCCGAACCCC AAGAATTGTT CCTGGGCACA AGGACTTAAT TTTCAGAAGA 2800
 10 TGTTCGGAAC CCCAAGAATT GTTCCTGGGC ACAAGGACTT AATTTTCAGA 2850
 AGATGCTTGA AGGCAGCATG TTCGTTAAGA GTCATCACCA CTCCCTAATC 2900
 TCAAGTACCC AGGGACACAA ACACTGCGGA AGGCCACAGG GTCCTCTGCA 2950
 TAGGAAAACC AGAGACCTTT GTTCACTTGT TTATCTGCTG ACCCTCCCTC 3000
 CACTATTGTC CTATGACCCT GCCAAATCCC CCTCTGTGAG AAACACCCAA 3050
 15 GAATGATCAA TAAAAAAAAA AAAAAAAAAA AAAAAAGTCG ACTCGAGAAT 3100
 TC 3102

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 783 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

	Met	Met	Cys	Gln	Lys	Phe	Tyr	Val	Val	Leu	Leu	His	Trp	Glu	Phe
	1				5					10				15	
25	Leu	Tyr	Val	Ile	Ala	Ala	Leu	Asn	Leu	Ala	Tyr	Pro	Ile	Ser	Pro
					20					25				30	
	Trp	Lys	Phe	Lys	Leu	Phe	Cys	Gly	Pro	Pro	Asn	Thr	Thr	Asp	Asp
					35					40				45	
30	Ser	Phe	Leu	Ser	Pro	Ala	Gly	Ala	Pro	Asn	Asn	Ala	Ser	Ala	Leu
					50					55				60	

	Lys Gly Ala Ser Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser	65	70	75
	Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val Phe His Cys Cys	80	85	90
5	Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu Thr Asp Asn	95	100	105
	Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser Val Phe	110	115	120
10	Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys Gly	125	130	135
	Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn	140	145	150
	Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu	155	160	165
15	Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser	170	175	180
	Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys	185	190	195
20	His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met	200	205	210
	Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu	215	220	225
	Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu	230	235	240
25	Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser	245	250	255
	Trp Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val	260	265	270
30	Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile	275	280	285
	Val Ser Ala Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser	290	295	300
	Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly	305	310	315

	Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe Thr Thr Gln Asp	
	320	325 330
	Val Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn	
	335	340 345
5	Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile Val Ser	
	350	355 360
	Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile Pro	
	365	370 375
10	Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr	
	380	385 390
	Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr	
	395	400 405
	Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr	
	410	415 420
15	Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu	
	425	430 435
	Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser	
	440	445 450
20	Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His	
	455	460 465
	Arg Cys Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr	
	470	475 480
	Ser Glu Pro Lys Thr Ala Ser Tyr Arg Glu Thr Ala Phe Met Asn	
	485	490 495
25	Val Phe Ser Ser Gln Ser Phe Tyr Tyr Leu Ala Ile Gln Cys Gly	
	500	505 510
	Phe Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr	
	515	520 525
30	Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn	
	530	535 540
	Val Lys Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser	
	545	550 555
	Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile	
	560	565 570

	Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu	575	580	585
	Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu L u Val Ser Asp	590	595	600
5	Leu Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu Asp	605	610	615
	Gly Leu Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu	620	625	630
10	Val Met Asp Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp Arg	635	640	645
	Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu	650	655	660
	Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg	665	670	675
15	Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser	680	685	690
	Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu	695	700	705
20	Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala	710	715	720
	Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys	725	730	735
	Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser	740	745	750
25	Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu	755	760	765
	Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly	770	775	780
30	Met Lys Trp	783		

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2868 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGGCCCCCCC TCGAAGTCGA CGGTATCGAT AAGCTTGATA TCGAATTCCG 50
 GCCGGGACAC AGGTGGGACA CTCTTTTAGT CCTCAATCCC TGGCGCGAGG 100
 CCACCCAAGG CAACGCAGGA CGCAGGGCGT TTGGGGACCA GGCAGCAGAC 150
 5 TGGGGCGGTA CCTGCGGAGA GCCACGCAAC TTCTCCAGGC CTCTGACTAC 200
 TTTGGAAACT GCCCGGGGCT GCGACATCAA CCCCTTAAGT CCCGGAGGCG 250
 GAAAGAGGGT GGGTTGGTTT GAAAGACACA AGGAAGAAAA ATGTGCTGTG 300
 GGGCGGGTTA AGTTTCCCAC CCTCTTCCCC CTTCCTGAGC AAATTAGAAA 350
 CAAAACAAAT AGAAAAGCCA GCCCTCCGGC CAACCAAAGC CCCAAGCGGA 400
 10 GCCCCAAGCG GAGCCCCAGC CGGAGCACTC CTTTAAAAGG ATTTGCAGCG 450
 GTGAGGAAAA AACCAGACCC GACCGAGGAA TCGTTCTGCA AATCCAGGTG 500
 TACACCTCTG AAGAAAGATG ATGTGTCAGA AATTCTATGT GGTTTTGTTA 550
 CACTGGGAAT TTCTTTATGT GATAGCTGCA CTTAACCTGG CATATCCAAT 600
 CTCTCCCTGG AAATTTAAGT TGTTTTGTGG ACCACCGAAC ACAACCGATG 650
 15 ACTCCTTTCT CTCACCTGCT GGAGCCCCAA ACAATGCCTC GGCTTTGAAG 700
 GGGGCTTCTG AAGCAATTGT TGAAGCTAAA TTTAATTCAA GTGGTATCTA 750
 CGTTCCTGAG TTATCCAAAA CAGTCTTCCA CTGTTGCTTT GGGAATGAGC 800
 AAGGTCAAAA CTGCTCTGCA CTCACAGACA AACTGAAGG GAAGACTG 850
 GCTTCAGTAG TGAAGGCTTC AGTTTTTCGC CAGCTAGGTG TAAACTGGGA 900
 20 CATAGAGTGC TGGATGAAAG GGGACTTGAC ATTATTCATC TGTCATATGG 950
 AGCCATTACC TAAGAACCCC TTCAAGAATT ATGACTCTAA GTCCATCTT 1000
 TTATATGATC TGCCTGAAGT CATAGATGAT TCGCCTCTGC CCCCACTGAA 1050
 AGACAGCTTT CAGACTGTCC AATGCAACTG CAGTCTTCGG GGATGTGAAT 1100
 GTCATGTGCC AGTACCCAGA GCCAAACTCA ACTACGCTCT TCTGATGTAT 1150
 25 TTGGAAATCA CATCTGCCGG TGTGAGTTTT CAGTCACCTC TGATGTCACT 1200
 GCAGCCCATG CTTGTTGTGA AACCCGATCC ACCCTTAGGT TTGCATATGG 1250

AAGTCACAGA TGATGGTAAT TTAAAGATTT CTTGGGACAG CCAAACAATG 1300

GCACCATTTC CGCTTCAATA TCAGGTGAAA TATTTAGAGA ATTCTACAAT 1350

TGTAAGAGAG GCTGCTGAAA TTGTCTCAGC TACATCTCTG CTGGTAGACA 1400

GTGTGCTTCC TGGATCTTCA TATGAGGTCC AGGTGAGGAG CAAGAGACTG 1450

5 GATGGTTCAG GAGTCTGGAG TGA CTGAGT TCACTCAAG TCTTTACCAC 1500

ACAAGATGTT GTGTATTTTC CACCCAAAAT TCTGACTAGT GTTGGATCGA 1550

ATGCTTCCTT TCATTGCATC TACAAAAACG AAAACCAGAT TGTCTCCTCA 1600

AAACAGATAG TTTGGTGGAG GAATCTAGCT GAGAAAATCC CTGAGATACA 1650

GTACAGCATT GTGAGTGACC GAGTTAGCAA AGTTACCTTC TCCAACCTGA 1700

10 AAGCCACCAG ACCTCGAGGG AAGTTTACCT ATGACGCAGT GACTGCTGC 1750

AATGAGCAGG CGTGCCATCA CCGCTATGCT GAATTATACG TGATCGATGT 1800

CAATATCAAT ATATCATGTG AAAC TGACGG GACTTAACT AAAATGACTT 1850

GCAGATGGTC ACCCAGCACA ATCCAATCAC TAGTGGGAAG CACTGTGCAG 1900

CTGAGGTATC ACAGGTGCAG CCTGTATTGT CTGATAGTC CATCTATTCA 1950

15 TCCTACGTCT GAGCCCCAAA CTGCGTCTTA CAGAGAGACG GCTTTTATGA 2000

ATGTGTTTTTC CAGCCAATCT TTCTATTATC TGGCTATACA ATGTGGATTTC 2050

AGGATCAACC ATTCTTTAGG TTCACCTGAC TCGCCACCAA CGTGTGTCCT 2100

TCCTGACTCC GTAGTAAAAC CACTACCTCC ATCTAACGTA AAAGCAGAGA 2150

TTACTGTAAA CACTGGATTA TTGAAAGTAT CTTGGGAAAA GCCAGTCTTT 2200

20 CCGGAGAATA ACCTTCAATT CCAGATTCGA TATGGCTTAA GTGGAAAAGA 2250

AATACAATGG AAGACACATG AGGTATTCGA TGCAAAGTCA AAGTCTGCCA 2300

GCCTGCTGGT GTCAGACCTC TGTGCAGTCT ATGTGGTCCA GGTTCGCTGC 2350

CGGCGGTGG ATGGACTAGG ATATTGGAGT AATTGGAGCA GTCCAGCCTA 2400

TACGCTTGTC ATGGATGTAA AAGTTCCTAT GAGAGGGCCT GAATTTTGGA 2450

25 GAAAAATGGA TGGGGACGTT ACTAAAAAGG AGAGAAATGT CACCTTGCTT 2500

TGGAAGCCCC TGACGAAAAA TGA CTCTG TGTAGTGTGA GGAGGTACGT 2550

GGTGAAGCAT CGTACTGCCC ACAATGGGAC GTGGTCAGAA GATGTGGGAA 2600
ATCGGACCAA TCTCACTTTC CTGTGGACAG AACCAGCGCA CACTGTTACA 2650
GTTCTGGCTG TCAATTCCTT CGGCGCTTCC CTTGTGAATT TTAACCTTAC 2700
CTTCTCATGG CCCATGAGTA AAGTGAGTGC TGTGGAGTCA CTCAGTGCTT 2750
5 ATCCCCTGAG CAGCAGCTGT GTCATCCTTT CCTGGACACT GTCACCTGAT 2800
GATTATAGTC TGTATATCT GGTTATTGAA TGGAAGATCC TTAATGAAGA 2850
TGATGGAATG AAGTGGCT 2868

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

15 GGGTTAAGTT TCCCACCC 18

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
20 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGTGGGAAA CTTAACCC 18

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
25 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGGATACAGT GGGATCCC 18

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
5 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCCCCGAGCAC TCCTTTAA 18

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

15 TTAAAGGAGT GCTCCCGC 18

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
20 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGCGGCCCT GTTAGATA 18

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTATACACCT CTGAAGAA 18

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
35 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTCTTCAGAG GTGTACAC 18

(2) INFORMATION FOR SEQ ID NO:17:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGCGAGGCT ACTTCTAT 18

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

15 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTCCCTGG AAATTTAA 18

20 (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTAAATTTCC AGGGAGAG 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATTTGAAGGA GTTAAGCC 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AATTTAATTC AAGTGGTA 18

10 (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TACCAGTTGA ATTAAATT 18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

25 GTATCACTTC ATAATATA 18

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GATGGTCAGG GTGAACTG 18

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
5 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CAGTTCACCC TGACCATC 18

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

15 GAGGCGAATG TGCGGATT 18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
20 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTAAATCTC CAAGGAGT 18

(2) INFORMATION FOR SEQ ID NO:28:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ACTCCTTGGA GATTTAAG 18

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
35 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AAGTCTTAAG CCAGACTT 18

(2) INFORMATION FOR SEQ ID NO:30:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCTAAGGCAC ATCCCAGC 18

(2) INFORMATION FOR SEQ ID NO:31:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCTGGGATGT GCCTTAGA 18

20 (2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
25 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGCAATGAAT TGACCCCC 18

(2) INFORMATION FOR SEQ ID NO:33:

- 30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TACTTCAGAG AAGTACAC 18

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTGTACTTCT CTGAAGTA 18

10 (2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GAATCACGGT AACTATCA 18

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

25 CAGCTGTCTC ATAATGTC 18

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GACATTATGA GACAGCTG 18

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TTCGTCAAGC CATCTGAT 18

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

His Gln Asn Leu Ser Asp Gly Lys

15

1 5 8

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

His Gln Asn Ile Ser Asp Gly Lys

1 5 8

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

His Gln Ser Leu Gly Thr Gln

30

1 5 7

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Val Ile Ser Ser His Leu Gly Gln
1 5 8

(2) INFORMATION FOR SEQ ID NO:43:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

10 Pro Lys Asn Ser Ser Met Ile Ser Asn Thr Pro
1 5 10 11

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
15 (B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10

20 (2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
25 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG 50

C 51

(2) INFORMATION FOR SEQ ID NO:46:

- 30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7127 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50
 TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100
 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150
 5 ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200
 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250
 ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300
 AAATGGCCCG CCTGGCATTG TGCCAGTAC ATGACCTTAT GGGACTTTCC 350
 TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400
 10 GGTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450
 TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500
 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550
 AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600
 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650
 15 CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700
 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750
 GTCTATAGGC CCACCCCTT GGCTTCGTGA GAACGCGGCT ACAATTAATA 800
 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850
 CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCAG GTCCAACTGC 900
 20 ACCTCGGTTT TATCGATATG CATTGGGGAA CCCTGTGCGG ATTCTTGTGG 950
 CTTTGGCCCT ATCTTTTCTA TGTCCAAGCT GTGCCCATCC AAAAAGTCCA 1000
 AGATGACACC AAAACCCTCA TCAAGACAAT TGTCACCAGG ATCAATGACA 1050
 TTTCACACAC GCAGTCAGTC TCCTCCAAAC AGAAAGTCAC CGGTTTGGAC 1100
 TTCATTCTTG GGCTCCACCC CATCCTGACC TTATCCAAGA TGGACCAGAC 1150
 25 ACTGGCAGTC TACCAACAGA TCCTCACCAG TATGCCTTCC AGAAACGTGA 1200
 TCCAAATATC CAACGACCTG GAGAACCTCC GGGATCTTCT TCACGTGCTG 1250

GCCTTCTCTA AGAGCTGCCA CTTGCCCTGG GCCAGTGGCC TGGAGACCTT 1300
GGACAGCCTG GGGGGTGTCC TGGAAGCTTC AGGCTACTCC ACAGAGGTGG 1350
TGGCCCTGAG CAGGCTGCAG GGGTCTCTGC AGGACATGCT GTGGCAGCTG 1400
GACCTCAGCC CTGGGTGCGG GGTACCGAC AAAACTCACA CATGCCCACC 1450
5 GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC 1500
CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTACATGC 1550
GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA 1600
CGTGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC 1650
AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG 1700
10 GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT 1750
CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG 1800
AACCACAGGT GTACACCCTG CCCCCATCCC GGGAAGAGAT GACCAAGAAC 1850
CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC 1900
CGTGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC 1950
15 CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC 2000
GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT 2050
GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC 2100
CGGGTAAATG AGTGCGACGG CCCTAGAGTC GACCTGCAGA AGCTTCTAGA 2150
GTCGACCTGC AGAAGCTTGG CCGCCATGGC CCAACTTGTT TATTGCAGCT 2200
20 TATAATGGTT ACAAATAAAG CAATAGCATC ACAAATTTC AATAAAGC 2250
ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAACTC ATCAATGTAT 2300
CTTATCATGT CTGGATCGAT CGGGAATTAA TTCGGCGCAG CACCATGGCC 2350
TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA 2400
AGAACCAGCT GTGGAATGTG TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC 2450
25 TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC 2500
CAGGTGTGGA AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA 2550

TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC 2600
CCGCCCCCTAA CTCCGCCCAG TTCCGCCCAT TCTCCGCCCC ATGGCTGACT 2650
AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT 2700
TCCAGAAGTA GTGAGGAGGC TTTTGTGGAG GCCTAGGCTT TTGCAAAAAG 2750
5 CTGTTAATTC GAACACGCAG ATGCAGTCGG GCGGGCGCGG TCCCAGGTCC 2800
ACTTCGCATA TTAAGGTGAC GCGTGTGGCC TCGAACACCG AGCGACCCTG 2850
CAGCGACCCG CTTAACAGCG TCAACAGCGT GCCGCAGATC TGATCAAGAG 2900
ACAGGATGAG GATCGTTTCG CATGATTGAA CAAGATGGAT TGCACGCAGG 2950
TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC 3000
10 AGACAATCGG CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG 3050
CGCCCGGTTT TTTTGTCAA GACCGACCTG TCCGGTGCCC TGAATGAACT 3100
GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GCGGTTCCCTT 3150
GCGCAGCTGT GCTCGACGTT GTCCTGAAG CGGGAAGGGA CTGGCTGCTA 3200
TTGGGCGAAG TGCCGGGGCA GGATCTCCTG TCATCTCACC TTGCTCCTGC 3250
15 CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGCGGCTG CATACGCTTG 3300
ATCCGGCTAC CTGCCCATTG GACCACCAAG CGAAACATCG CATCGAGCGA 3350
GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG ATCTGGACGA 3400
AGAGCATCAG GGGCTCGCGC CAGCCGAACT GTTCGCCAGG CTCAAGGCGC 3450
GCATGCCCCG CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG 3500
20 CCGAATATCA TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG 3550
CCGGCTGGGT GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG 3600
ATATTGCTGA AGAGCTTGGC GGCGAATGGG CTGACCGCTT CCTCGTGCTT 3650
TACGGTATCG CCGCTCCCGA TTCGCAGCGC ATCGCCTTCT ATCGCCTTCT 3700
TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC 3750
25 GACGCCAAC CTGCCATCAC GAGATTTTGA TTCCACCGCC GCCTTCTATG 3800
AAAGGTTGGG CTTCCGAATC GTTTTCCGGG ACGCCGGCTG GATGATCCTC 3850

CAGCGCGGGG ATCTCATGCT GGAGTTCTTC GCCCACCCCG GGAGATGGGG 3900
GAGGCTAACT GAAACACGGA AGGAGACAAT ACCGGAAGGA ACCCGCGCTA 3950
TGACGGCAAT AAAAAGACAG AATAAACGC ACGGGTGTTG GGTCGTTTGT 4000
TCATAAACGC GGGGTTCGGT CCCAGGGCTG GCACTCTGTC GATACCCAC 4050
5 CGAGACCCCA TTGGGGCCAA TACGCCCGCG TTTCTTCCTT TTCCCCACCC 4100
CAACCCCAAA GTTCGGGTGA AGGCCAGGG CTCGCAGCCA ACGTCGGGGC 4150
GGCAAGCCCG CCATAGCCAC GGGCCCCGTG GGTTAGGGAC GGGGTCCCCC 4200
ATGGGGAATG GTTTATGGTT CGTGGGGGTT ATTCTTTTGG GCGTTGCGTG 4250
GGGTCAGGTC CACGACTGGA CTGAGCAGAC AGACCCATGG TTTTGGATG 4300
10 GCCTGGGCAT GGACCGCATG TACTGGCGCG ACACGAACAC CGGGCGTCTG 4350
TGGCTGCCAA ACACCCCGA CCCCCAAAA CCACCGCGCG GATTTCTGGC 4400
GCCGCCGGAC GAACTAAACC TGA CTACGGC ATCTCTGCCC CTTCTTCGCT 4450
GGTACGAGGA GCGCTTTTGT TTTGTATTGG TCACCACGGC CGAGTTTCCG 4500
CGGGACCCCG GCCAGGGCAC CTGTCCTACG AGTTGCATGA TAAAGAAGAC 4550
15 AGTCATAAGT GCGGCGACGA TAGTCATGCC CCGCGCCAC CGGAAGGAGC 4600
TGA CTGGGTT GAAGGCTCTC AAGGGCATCG GTCGAGCGGC CGCATCAAAG 4650
CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTTGGT 4700
GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC 4750
CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTGCGCCG CTTTCCCCGT 4800
20 CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG 4850
GCACCTCGAC CCCAAAAAAC TTGATTGTTG TGATGGTTCA CGTAGTGGGC 4900
CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA GTCCACGTTT 4950
TTTAATAGTG GACTCTTGTT CCAA CTGGA ACAACTCA ACCCTATCTC 5000
GGGCTATTCT TTTGATTAT AAGGGATTTT GCCGATTTCG GCCTATTGGT 5050
25 TAAAAAATGA GCTGATTAA CAAAAATTA ACGCGAATT TAACAAAATA 5100
TTAACGTTTA CAATTTTATG GTGCAGGCCT CGTGATACGC CTATTTTAT 5150

AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT 5200

CGGGGAAATG TGC GCGGAAC CCCTATTTGT TTATTTTCT AAATACATTC 5250

AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT 5300

ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT 5350

5 CCCTTTTGTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT 5400

GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTACA 5450

TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA 5500

GAACGTTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT 5550

ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT 5600

10 ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT 5650

ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG 5700

TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG 5750

AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT 5800

CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC 5850

15 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAATA TTAAGTGGCG 5900

AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG 5950

GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 6000

TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG 6050

CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG 6100

20 ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT 6150

AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT 6200

ATATACTTTA GATTGATTTA AAACCTTCAAT TTTAATTTAA AAGGATCTAG 6250

GTGAAGATCC TTTTGTGATA TCTCATGACC AAAATCCCTT AACGTGAGTT 6300

TTCGTTCCAC TGAGCGTCAG ACCCGTAGA AAAGATCAAA GGATCTTCTT 6350

25 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAACCA 6400

CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT 6450

TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC 6500
 TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT 6550
 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA 6600
 TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG 6650
 5 CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG 6700
 CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG 6750
 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6800
 GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG 6850
 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT 6900
 10 TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAC GCCAGCTGGC 6950
 ACGACAGGTT TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT 7000
 GTGAGTTACC TCACTCATTG GGCACCCCAG GCTTTACACT TTATGCTTCC 7050
 GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA 7100
 ACAGCTATGA CCATGATTAC GAATTAA 7127

15 (2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 397 amino acids
- (B) TYPE: Amino Acid
- (D) TOPOLOGY: Linear

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Met	His	Trp	Gly	Thr	Leu	Cys	Gly	Phe	Leu	Trp	Leu	Trp	Pro	Tyr
1				5					10					15
Leu	Phe	Tyr	Val	Gln	Ala	Val	Pro	Ile	Gln	Lys	Val	Gln	Asp	Asp
			20						25					30
25	Thr	Lys	Thr	Leu	Ile	Lys	Thr	Ile	Val	Thr	Arg	Ile	Asn	Asp
			35						40					45
Ser	His	Thr	Gln	Ser	Val	Ser	Ser	Lys	Gln	Lys	Val	Thr	Gly	Leu
			50						55					60
30	Asp	Phe	Ile	Pro	Gly	Leu	His	Pro	Ile	Leu	Thr	Leu	Ser	Lys
			65						70					75

	Asp	Gln	Thr	Leu	Ala	Val	Tyr	Gln	Gln	Ile	Leu	Thr	Ser	Met	Pro	80	85	90
	Ser	Arg	Asn	Val	Ile	Gln	Ile	Ser	Asn	Asp	Leu	Glu	Asn	Leu	Arg	95	100	105
5	Asp	Leu	Leu	His	Val	Leu	Ala	Phe	Ser	Lys	Ser	Cys	His	Leu	Pro	110	115	120
	Trp	Ala	Ser	Gly	Leu	Glu	Thr	Leu	Asp	Ser	Leu	Gly	Gly	Val	Leu	125	130	135
10	Glu	Ala	Ser	Gly	Tyr	Ser	Thr	Glu	Val	Val	Ala	Leu	Ser	Arg	Leu	140	145	150
	Gln	Gly	Ser	Leu	Gln	Asp	Met	Leu	Trp	Gln	Leu	Asp	Leu	Ser	Pro	155	160	165
	Gly	Cys	Gly	Val	Thr	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	170	175	180
15	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	185	190	195
	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	200	205	210
20	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	215	220	225
	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	230	235	240
	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	245	250	255
25	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	260	265	270
	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	275	280	285
30	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	290	295	300
	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	305	310	315
	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	320	325	330

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 335 340 345
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 350 355 360
 5 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
 365 370 375
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 380 385 390
 Leu Ser Leu Ser Pro Gly Lys
 10 395 397

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 249 amino acids

(B) TYPE: Amino Acid

15 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
 20 20 25 30
 Gly Tyr Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 35 40 45
 Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
 50 55 60
 25 Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
 65 70 75
 Ile Gly Thr Ala Tyr Met Glu Leu Ser Arg Leu Ser Ser Asp Asp
 80 85 90
 Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Tyr Tyr Gly Ser Ser
 30 95 100 105
 Ala Tyr His Arg Gly Ser Tyr Tyr Met Asp Val Trp Gly Arg Gly
 110 115 120
 Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Thr Gly Gly Gly
 125 130 135

Gly Ser Gly Gly Gly Gly Ser Ser Glu Leu Thr Gln Asp Pro Ala
 140 145 150
 Val Ser Val Ala Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly
 155 160 165
 5 Asp Ser Leu Arg Ser Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro
 170 175 180
 Gly Gln Ala Pro Val Leu Val Ile Tyr Gly Lys Asn Asn Arg Pro
 185 190 195
 10 Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Ser Ser Gly Asn Thr
 200 205 210
 Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp Glu Ala Asp
 215 220 225
 Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His Val Val Phe
 230 235 240
 15 Gly Gly Gly Thr Lys Leu Thr Val Leu
 245 249

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 250 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15
 25 Glu Ser Leu Lys Ile Ser Cys Gln Gly Ser Gly Phe Thr Phe Ser
 20 25 30
 Ser Tyr Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 35 40 45
 30 Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr
 50 55 60
 Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser
 65 70 75
 Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp
 80 85 90

	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Asp	Arg	Val	Val	Val	Pro	Ala	
						95				100					105	
	Thr	Ser	Leu	Arg	Gly	Gly	Met	Asp	Val	Trp	Gly	Gln	Gly	Thr	Thr	
					110					115					120	
5	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	
					125					130					135	
	Gly	Gly	Gly	Gly	Ser	Gln	Ser	Val	Leu	Thr	Gln	Pro	Ala	Ser	Val	
					140					145					150	
	Ser	Gly	Ser	Pro	Gly	Gln	Ser	Ile	Thr	Ile	Ser	Cys	Thr	Gly	Thr	
10					155					160					165	
	Ser	Ser	Asp	Val	Gly	Gly	Tyr	Asn	Tyr	Val	Ser	Trp	Tyr	Gln	Gln	
					170					175					180	
	His	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Met	Ile	Tyr	Glu	Gly	Ser	Lys	
					185					190					195	
15	Arg	Pro	Ser	Gly	Val	Ser	Asn	Arg	Phe	Ser	Gly	Ser	Lys	Ser	Gly	
					200					205					210	
	Ser	Thr	Ala	Ser	Leu	Thr	Ile	Ser	Gly	Leu	Gln	Ala	Glu	Asp	Glu	
					215					220					225	
	Ala	Asp	Tyr	Tyr	Cys	Ser	Ser	Tyr	Thr	Thr	Arg	Ser	Thr	Arg	Val	
20					230					235					240	
	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu						
					245					250						

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 241 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

30	Gln	Val	Arg	Leu	Gln	Gln	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	
	1				5					10					15	
	Arg	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asp	
					20					25					30	
	Asp	Tyr	Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	
					35					40					45	

	Glu Trp Val Ser Gly Met Thr Trp Asn Ser Gly Ser Ile Gly Tyr	
	50	55 60
	Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala	
	65	70 75
5	Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp	
	80	85 90
	Thr Ala Val Tyr Tyr Cys Ala Arg Glu Pro His Asn Thr Asp Ala	
	95	100 105
10	Phe Asp Ile Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Gly	
	110	115 120
	Gly Gly Gly Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp	
	125	130 135
	Val Val Met Thr Gln Ser Pro Ser Phe Leu Ser Ala Phe Val Gly	
	140	145 150
15	Asp Thr Ile Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Tyr Asn	
	155	160 165
	Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu	
	170	175 180
	Leu Ile Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg	
20	185	190 195
	Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser	
	200	205 210
	Ser Leu Gln Pro Glu Asp Phe Gly Thr Tyr Tyr Cys Gln Gln Leu	
	215	220 225
25	Ile Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile	
	230	235 240
	Lys	
	241	

(2) INFORMATION FOR SEQ ID NO:51:

- 30 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 894 amino acids
 - (B) TYPE: Amino Acid
 - (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

	Met	Met	Cys	Gln	Lys	Phe	Tyr	Val	Val	Leu	Leu	His	Trp	Glu	Phe	
	1				5					10				15		
	Leu	Tyr	Val	Ile	Ala	Ala	Leu	Asn	Leu	Ala	Tyr	Pro	Ile	Ser	Pro	
				20						25				30		
5	Trp	Lys	Phe	Lys	Leu	Phe	Cys	Gly	Pro	Pro	Asn	Thr	Thr	Asp	Asp	
				35						40				45		
	Ser	Phe	Leu	Ser	Pro	Ala	Gly	Ala	Pro	Asn	Asn	Ala	Ser	Ala	Leu	
				50						55				60		
10	Lys	Gly	Ala	Ser	Glu	Ala	Ile	Val	Glu	Ala	Lys	Phe	Asn	Ser	Ser	
				65						70				75		
	Gly	Ile	Tyr	Val	Pro	Glu	Leu	Ser	Lys	Thr	Val	Phe	His	Cys	Cys	
				80						85				90		
	Phe	Gly	Asn	Glu	Gln	Gly	Gln	Asn	Cys	Ser	Ala	Leu	Thr	Asp	Asn	
				95						100				105		
15	Thr	Glu	Gly	Lys	Thr	Leu	Ala	Ser	Val	Val	Lys	Ala	Ser	Val	Phe	
				110						115				120		
	Arg	Gln	Leu	Gly	Val	Asn	Trp	Asp	Ile	Glu	Cys	Trp	Met	Lys	Gly	
				125						130				135		
	Asp	Leu	Thr	Leu	Phe	Ile	Cys	His	Met	Glu	Pro	Leu	Pro	Lys	Asn	
20				140						145				150		
	Pro	Phe	Lys	Asn	Tyr	Asp	Ser	Lys	Val	His	Leu	Leu	Tyr	Asp	Leu	
				155						160				165		
	Pro	Glu	Val	Ile	Asp	Asp	Ser	Pro	Leu	Pro	Pro	Leu	Lys	Asp	Ser	
				170						175				180		
25	Phe	Gln	Thr	Val	Gln	Cys	Asn	Cys	Ser	Leu	Arg	Gly	Cys	Glu	Cys	
				185						190				195		
	His	Val	Pro	Val	Pro	Arg	Ala	Lys	Leu	Asn	Tyr	Ala	Leu	Leu	Met	
				200						205				210		
30	Tyr	Leu	Glu	Ile	Thr	Ser	Ala	Gly	Val	Ser	Phe	Gln	Ser	Pro	Leu	
				215						220				225		
	Met	Ser	Leu	Gln	Pro	Met	Leu	Val	Val	Lys	Pro	Asp	Pro	Pro	Leu	
				230						235				240		
	Gly	Leu	His	Met	Glu	Val	Thr	Asp	Asp	Gly	Asn	Leu	Lys	Ile	Ser	
				245						250				255		

	Trp Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val	
	260	265 270
	Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile	
	275	280 285
5	Val Ser Ala Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser	
	290	295 300
	Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly	
	305	310 315
10	Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe Thr Thr Gln Asp	
	320	325 330
	Val Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn	
	335	340 345
	Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile Ile Ser	
	350	355 360
15	Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile Pro	
	365	370 375
	Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr	
	380	385 390
20	Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr	
	395	400 405
	Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr	
	410	415 420
	Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu	
	425	430 435
25	Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser	
	440	445 450
	Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His	
	455	460 465
30	Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr	
	470	475 480
	Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu	
	485	490 495
	Cys Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp	
	500	505 510

	Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr		
	515	520	525
	Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn		
	530	535	540
5	Val Lys Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser		
	545	550	555
	Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile		
	560	565	570
10	Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu		
	575	580	585
	Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu Leu Val Ser Asp		
	590	595	600
	Leu Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu Asp		
	605	610	615
15	Gly Leu Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu		
	620	625	630
	Val Met Asp Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp Arg		
	635	640	645
20	Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu		
	650	655	660
	Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg		
	665	670	675
	Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser		
	680	685	690
25	Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu		
	695	700	705
	Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala		
	710	715	720
30	Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys		
	725	730	735
	Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser		
	740	745	750
	Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu		
	755	760	765

	Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly	
	770	775 780
	Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile	
	785	790 795
5	His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr	
	800	805 810
	Pro Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Gly	
	815	820 825
10	Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly Leu	
	830	835 840
	Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu	
	845	850 855
	Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp	
	860	865 870
15	Asp Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu	
	875	880 885
	Asn Phe Gln Lys Arg Thr Asp Thr Leu	
	890	894

WHAT IS CLAIMED IS:

1. Isolated WSX receptor.
2. The WSX receptor of claim 1 comprising an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of mature human WSX receptor variant 6.4 shown in Figs 2A-B; (b)
5 the amino acid sequence of mature human WSX receptor variant 12.1 shown in Figs. 2A-B; and (c) the amino acid sequence of mature human WSX receptor variant 13.2 shown in Figs 2A-B.
3. The WSX receptor of claim 2 which is mature human WSX receptor variant 13.2.
4. The WSX receptor of claim 1 which is WSX receptor extracellular domain (ECD).
5. The WSX receptor ECD of claim 4 which is conjugated with, or fused to, a molecule which
10 increases the serum half-life thereof.
6. The WSX receptor ECD of claim 5 which is conjugated with polyethylene glycol (PEG).
7. A composition comprising the WSX receptor ECD of claim 4 and a physiologically acceptable carrier.
8. The composition of claim 7 further comprising WSX ligand.
9. The WSX receptor of claim 1 which is chimeric WSX receptor.
15
10. The chimeric WSX receptor of claim 9 comprising a WSX receptor amino acid sequence fused to an immunoglobulin sequence.
11. The chimeric WSX receptor of claim 10 comprising a fusion of a WSX receptor extracellular domain sequence to an immunoglobulin constant domain sequence.
- 20 12. The chimeric WSX receptor of claim 11 wherein said constant domain sequence is that of an immunoglobulin heavy chain.
13. A method for identifying a molecule which binds to the WSX receptor comprising exposing the WSX receptor to a molecule suspected of binding thereto and determining binding of the molecule to the WSX receptor.
- 25 14. A method for identifying a molecule which activates the WSX receptor comprising exposing the WSX receptor to a molecule suspected of being capable of activating the WSX receptor and measuring activation of the WSX receptor.
15. A method for purifying a molecule which binds to the WSX receptor comprising adsorbing the molecule to WSX receptor immobilized on a solid phase and recovering the molecule from the immobilized
30 WSX receptor.
16. An antibody that specifically binds to the WSX receptor of claim 1.
17. The antibody of claim 16 which is an agonist antibody.
18. The antibody of claim 17 which has an IC₅₀ in a KIRA ELISA of about 0.5µg/ml or less.
19. The antibody of claim 16 which is a neutralizing antibody.

20. The antibody of claim 16 which is a human or humanized antibody.
21. The antibody of claim 16 which is an antibody fragment.
22. The antibody fragment of claim 21 which is an F(ab')₂.
23. A composition comprising the antibody of claim 16 and a physiologically acceptable carrier.
- 5 24. The composition of claim 23 further comprising a cytokine.
25. A method for activating the WSX receptor comprising exposing the WSX receptor to an amount of the antibody of claim 17 which is effective for activating the WSX receptor.
26. A method for enhancing proliferation or differentiation of a cell comprising the WSX receptor comprising exposing the cell to an amount of the antibody of claim 17 which is effective for enhancing
10 proliferation or differentiation of the cell.
27. The method of claim 26 wherein the cell is a CD34+ cell.
28. A method for determining the presence of a WSX receptor comprising exposing a test sample suspected of containing the WSX receptor to the antibody of claim 16 and determining binding of said antibody to the test sample.
- 15 29. An isolated nucleic acid molecule encoding the WSX receptor of claim 1.
30. An isolated nucleic acid molecule encoding the WSX receptor ECD of claim 4.
31. An isolated nucleic acid molecule encoding the chimeric WSX receptor of claim 9.
32. The isolated nucleic acid molecule of any one of claims 29-31 further comprising a promoter operably linked to the nucleic acid molecule.
- 20 33. An expression vector comprising the nucleic acid molecule of any one of claims 29-31 operably linked to control sequences recognized by a host cell transformed with the vector.
34. A host cell comprising the vector of claim 33.
35. A process of using a nucleic acid molecule encoding the WSX receptor to effect production of the WSX receptor comprising culturing the host cell of claim 34.
- 25 36. A method for enhancing proliferation or differentiation of a cell comprising the WSX receptor comprising exposing the cell to an amount of WSX ligand which is effective for enhancing proliferation or differentiation of the cell.
37. The method of claim 36 wherein the WSX receptor is the WSX receptor variant 13.2.
38. The method of claim 36 wherein the cell is a hematopoietic progenitor cell.
- 30 39. The method of claim 36 wherein the WSX ligand is OB protein.
40. The method of claim 36 wherein the WSX ligand is an anti-WSX receptor agonist antibody.
41. The method of claim 36 which enhances proliferation or differentiation of lymphoid blood
cell lineages.
42. The method of claim 36 which enhances proliferation or differentiation of myeloid blood cell
35 lineages.

43. The method of claim 36 which enhances proliferation or differentiation of erythroid blood cell lineages.
44. The method of claim 36 further comprising exposing the cell to a further cytokine.
45. The method of claim 44 wherein the further cytokine is a lineage-specific cytokine.
- 5 46. The method of claim 36 wherein the cell is present in a mammal.
47. The method of claim 46 wherein the mammal is a human.
48. The method of claim 46 wherein the mammal is suffering from, or is expected to suffer from, decreased blood cell levels.
49. The method of claim 48 wherein the decreased blood cell levels are caused by chemotherapy, radiation therapy, or bone marrow transplantation therapy.
- 10 50. A method for repopulating blood cells in a mammal comprising administering to the mammal a therapeutically effective amount of a WSX ligand.
51. The method of claim 50 wherein the blood cells are erythroid cells.
52. The method of claim 50 wherein the blood cells are myeloid cells.
- 15 53. The method of claim 50 wherein the blood cells are lymphoid cells.
54. The method of claim 50 comprising administering a further cytokine to the mammal in an amount which leads to a synergistic repopulation of the blood cells in the mammal.
55. A pharmaceutical composition comprising WSX ligand, a further cytokine, and a physiologically acceptable carrier.
- 20 56. An article of manufacture, comprising:
a container;
a label on the container; and
a composition comprising an active agent contained within the container; wherein the composition is effective for repopulating blood cells in a mammal, the label on the container indicates that the composition can be used for repopulating blood cells in a mammal and the active agent in the composition is a WSX ligand.
- 25 57. The article of manufacture of claim 56 comprising a further container which holds a further cytokine.
58. An article of manufacture, comprising:
a container;
a label on the container; and
30 a composition comprising an active agent contained within the container; wherein the composition is effective for decreasing body weight or fat-depot weight or decreasing food intake in an obese mammal, the label on the container indicates that the composition can be used for treating obesity in a mammal and the active agent in the composition is an agonist anti-WSX receptor antibody.

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sites: std
length: 4102 (circular)

pleI
hinfI
xhoI salI
paer7I taqI aluI
ecoRI taqI hincII/hindII tru9I
apoI avai accI acII mseI
1 GAATTCCTGA GTCGACGGCG GCGGTTAAAG CTCTCGTGGC ATTATCCTTC AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTAGAGGA
CTTAAGAGCT CAGCTGCCGC CCGCAATTTC GAGAGCACCG TAATAGGAAG TCACCCCGAT AACCTGACTG AAAAGAATAC GACCTACAC GGAATCTCCT

rsalI
mspI
101 TTATGGGTGT ACTTCTCTGA AGTAAGATGA TTGTCAAAA ATTCTGTGTG GTTTGTGTAC ATTGGGAATT TATTATGTG ATAACTGCGT TAAACTTGTG
AATACCCACA TGAAGAGACT TCATTCTACT AAACAGTTTT TAAGACACAC CAAACAATG TAACCCCTAA ATAAATACAC TATTGACGCA AATTGAACAG

1 M I C Q K F C V V L L H W E F I Y V I T A F N L S

nlalII
sphi
nspi
nspHI
201 ATATCCCAATT ACTCCTTGA GATTAAAGTT GTCTTGCATG CCACCAAAAT CAACCTATGA CTACTTCCTT TTGCCTGCTG GACTCTCAA GAATACTTCA
TATAGGTTAA TGAGGAACCT CTAATTTCAA CAGAACGTAC GGTGGTTTAA GTTGATATCT GATGAAGGAA AACGGACGAC CTGAGAGTTT CTTATGAAGT

26 Y P I T P W R F K L S C M P P N S T Y D Y F L L P A G L S K N T S

taqI
sfuI
bstBI
bsiCI
asuII
pvuII
nspBII
bsmAI aluI
301 AATTCGAATG GACATTATGA GACAGCTGTT GAACCTAAGT TTAATTCAAG TGGTACTCAC TTTTCTAACT TATCCAAAAC AACTTTCCAC TGTGCTTTC
TTAAGCTTAC CTGTAATACT CTGTCGACAA CTGCGATTCA AATTAAGTTC ACCATGATG AAAAGATTGA ATAGGTTTGT TTGAAGGTG ACACGAAAG

59 N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R

apoI
sfanI
401 GGAGTGAGCA AGATAGAAC TGCTCCTTAT GTGCAGACAA CATTGAAGGA AAGACATTGT TTTCAACAGT AAATCTTTA GTTTTCAAC AAATAGATGC
CCTCACTCGT TCTATCTTTG ACGAGGAATA CACGCTCTGT GTAACTTCTT TCTGTAAAC AAAGTTGTCA TTTAAGAAAT CAAAAGTTG TTTATCTACG

93 S E Q D R N C S L C A D N I E G K T F V S T V N S L V F Q Q I D A

FIG. 1A

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      xmnI      tfII      hinfI      pleI      tru9I      msel      asp700      sau96I
      xmnI      tfII      hinfI      pleI      tru9I      msel      asp700      avall      asuI
501 A A A C T G G A C A T A C A G T G C T G G C T A A A A G G A G A C T T A A A A T T A T T C A T C T G T A T G T G G A G T C A T T A T T T A A G A A T C T A T T C A G G A A T T A T A A C T A A G
126 N W N I Q C W L K G D L K L F I C Y V E S L F K N L F R N Y N Y K

      bsrI      bsmAI      msel      tru9I      hinfI      hphI      nlaIII      rcal      bspHI
      bsrI      bsmAI      msel      tru9I      hinfI      hphI      nlaIII      rcal      bspHI
601 G T C C A T C T T T A T A T G T T C T G C T G A A G T G T T A G A A G A T T C A C T C T G G T T C C C C A A A A G G C A G T T T T C A G A T G T T C A C T G C A A T T G C A G T G T C A T G
159 V H L L Y V L P E V L E D S P L V P Q K G S F Q M V H C N C S V H E

      tfII      hinfI      mnlI      bslI      nlaIII      hphI      maelII
      tfII      hinfI      mnlI      bslI      nlaIII      hphI      maelII
701 A A T G T T G T G A T G T C T T G T G C C T G T G C C A A C A G C A C A C T C A C G A C A C T C T C T T A T G T G T T G A A A T C A C A T C T G G T G G A G T A A T T T T C C A G T C A C C
193 C C E C L V P V P T A K L N D T L L M C L K I T S G G V I F Q S P

      bslI      sau3AI      mboI/ndeII(dam-)      dpnI(dam+)      dpnII(dam-)      alwI(dam-)      ndeI
      bslI      sau3AI      mboI/ndeII(dam-)      dpnI(dam+)      dpnII(dam-)      alwI(dam-)      ndeI
801 T C T A A T G T C A G T C A G C C C C A T A A T A T G T G T G A A G C C T G A T C C A C C A T T A G G T T T G C A T A T G G A A A T C A C A G A T G C T A T G A T G C T A T G G A A T C A C A G A T G C T A
226 L M S V Q P I N M V K P D P P L G L H M E I T D D G N L K I S W S

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FIG. 1B

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rsal
csp6I
nlaIV
kpnI
hgiCI
banI
asp718
bslI
acc65I
901 AGCCACCAT TGGTACCATT TCCACTTCAA TATCAAGTGA AATATTCTGA GAATTCTACA ACAGTTATCA GAGAAGCTGA CAAGATTGTC TCAGCTACAT
TCGGGTGGTA ACCATGGTAA AGGTGAAGTT ATAGTTCACT TTATAAGTCT CTTAAGATGT TGTCRAATAGT CTCCTTCGACT GTTCTAACAG AGTCGATGTA
259 S P L V P F P L Q Y Q V K Y S E N S T V I R E A D K I V S A T S
^begin12u
ecorI
spol
sspl
apoI
drdI
ddeI
326 R V F T T Q D V I Y F P P K I L T S V G S N V S F H C I Y K K E N
sfaNI
1101 TCGTGCTTTT ACCACACAAG ATGTCATATA CTTTCCACCT AAATTTCTGA CAAGTGTTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAARAC
AGCACAGAAA TGGTGTGTTT TACAGTATAT GAAAGGTGGA TTTTAAGACT GTTCACAACC CAGATTACAA AGAAAGTGA CGTAGATATT CTTCCCTTTG

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FIG. 1C

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nlaIII
sau3AI
mboI/ndeII[dam-]
dpmI[dam+]
dpmII[dam-]
bclI[dam-]
maeIII
1201 AAGATTGTTT CCTCAAGA GATTGTTGG TGGATGAATT TAGCTGAGAA AATTCCTCAA AGCCAGTATG ATGTTGTGAG TGATCATGTT AGCAAGTTA
TTCTAACAG GAGCTTTCT CTAACAACC ACCTACTTAA ATCGACTCTT TTAAGGAGTT TCGGTCTATC TACAACACTC ACTAGTACAA TCGTTTCAAT
359 K I V P S K E I V W W M N L A E K I P Q S Q Y D V V S D H V S K V T
apoI ddeI
fokI aluI
1301 CTTTTTCAA TCTGAATGAA ACCAAACCTC GAGGAAGTT TACCTATGAT GCAGTGTACT GCTGCAATGA ACATGATGC CATCATCGCT ATGCTGAATT
GAAAAGATT AGACTTACTT TGGTTTGGAG CTCCTTTCAA ATGGATCTA CGTCACATGA CGACGTACT TGTACTTACG GTAGTACGA TAGGACTTAA
393 F F N L N E T K P R G K F T Y D A V Y C C N E H E C H H R Y A E L
rsal fnu4HI bsmI
sfanI csp6I bbvI
1401 ATATGTGATT GATGTCATA TCAATATCTC ATGTGAACT GATGGTACT TAACTAAAAT GACTGCGAGA ATTCATCCCA GTACAATCCA GTCACTTGGC
TATACACTAA CTACAGTAT AGTTATAGAG TACACTTGA CTACCATGA ATTGATTITA CTGAACGTCT ACCAGTTGGT CATGTAGGT CAGTGAACGC
426 Y V I D V N I N I S C E T D G Y L T K M T C R W S T S T I Q S L A
nlaIII
rsal csp6I
trn9I mseI
1501 GAAAGCACTT TGCATTTGAG GTATCATAGG AGCAGCCTTT ACTGTTCTGA TATTCATCT ATTCATCCCA TATCTGAGCC CAAAGATTGC TATTGCGAGA
CTTTCGTCAA ACGTTAACTC CATAGTATCC TCGTCGGAAA TGACAAGACT ATAAGGTAGA TAAGTAGGT ATAGACTCGG GTTCTAACG ATAAACGTCT
459 E S T L Q L R S S L Y C S D I P S I H P I S E P K D C Y L Q S
fnu4HI
bbvI
fokI ddeI

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FIG. 1D

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sau3AI
mboI/ndelII(dam-)
dpmI(dam+)
dpmII(dam-)
alwI(dam-)
rmaI
pleI
1601 GTGATGGTTT TTATGAATGC ATTTCCAGC CAATCTTCCT ATTATCTGGC TACCAATGT GGATTAGGAT CAATCACTCT CTAGGTTCCAC TTGACTCTCC
CACTACCAAA AATACCTAGC TAAAGGTGC GTTAGAGGA TAATAGACCG ATGTGTACA CCTAATCCTA GTTACTGAGA GATCCAAGTG AACTGAGAGG
493 D G F Y E C I F Q P I F L L S G Y T M W I R I N H S L G S L D S P
^begin13-2
ppu10I
nslI/avaIII
bsmI
mboII
draIII
bsaBI(dam-)
rmaI
pleI
1701 ACCAACATGT GTCCTTCCTG ATTCTGCGT GAAGCCACTG CCTCCATCCA GTGTGAAGC AGAAATTACT ATAAACATTG GATTATTGAA AATATCTTGG
TGGTTGTACA CAGGAAGGAC TAAGACACCA CTTCCGTGAC GGAGGTAGGT CACACTTTCG TCTTTAATGA TATTGTAACT CTAATAACTT TTATAGAACC
526 P T C V L P D S V V K P L P P S S V K A E I T I N I G L L K I S W
tfII
hinfI
1801 GAAAGCCAG TCTTTCAGA GAATAACCTT CAATTCAGA TCGCTATGG TTAAAGTGA AAAGAAGTAC AATGGAAGAT GTATGAGGT TATGATGCAA
CTTTCCGTC AGAAGGTCT CTATTGGAA GTTAAGGTCT AAGCATACC AAATTCACCT TTTCTTCATG TTACCTTCTA CATACTCCAA ATACTACGTT
559 E K P V F P E N N L Q F Q I R Y G L S G K E V Q W K M Y E V Y D A K
bsrI
xcmI
1901 AATCAAAATC TGTAGTCTC CCAGTTCAG ACTTGTGTC AGTCTATGCT GTTCAGGTGC GCTGTAAGAG GCTAGATGGA CTGGATATT GGAGTAATTG
TTAGTTTAG ACAGTCAGAG GGTCAGGTC TGAACACACG TCAGATACGA CAAGTCCACG CGACATTCTC CGATCTACCT GACCCTATAA CCTCATTAA
593 S K S V S L P V P D L C A V Y A V Q V R C K R L D G L G Y W S N W
bsmAI bsrI
hinPI
rmaI
hhaI/cfoI mnlI maeI bsrI
2001 GAGCAATCCA GCCTACACAG TTGTCATGGA TATAAAGTT CCTATGACAG GACCTGAATT TTGGACAATA ATTATGGAG ATACTATGAA AAAGGAGAAA
CTCGTTAGGT CGGATGTGC AACAGTACCT ATATTTTCAA GGATACCTC CTGGACTTAA AACCTTAT TAATACCTC TATGATACTT TTTCCTCTT
626 S N P A Y T V V M D I K V P M R G P E F W R I I N G D T M K E K

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FIG. 1E

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2101 AATGTCACCT TACTTTGGAA GCCCTGATG AAAAATGACT CATGTGCAG TGTCAGAGA TATGTGATA ACCATCATAC TTCTTGCAAT GGAACATGGT nlaIII
 TTACAGTGA ATGAACCTT CGGGACTAC TTTTACTGA GTAACAGTC ACAAGTCTCT ATACACTATT TGGTAGTATG AAGCAGTTA CCTTGATCCA
 659 N V T L L W K P L M K N D S L C S V Q R Y V I N H T S C N G T W S
 maeIII pleI
 hinfI
 2201 CAGAAGATGT GGGAATCAC ACGAATTCA CTTTCCTGTG GACAGAGCAA GCACATACTG TTACGGTTCT GCCATCAAT TCAATTGGTG CTTCTGTTGC muniI
 GTCCTTACA CCCTTAGTG TGCTTTAAGT GAAAGGACAC CTGTCTCGTT CGGTATGAC AATGCCAAGA CCGTAGTTA AGTTAACCCAC GAAGACAACG
 693 E D V G N H T K F T F L W T E Q A H T V T V L A I N S I G A S V A
 mboII
 apoI
 maeIII
 cfrI
 haeIII/palI
 mscI/balI
 haeI
 eaeI
 2301 AAATTTAAT TTAACCTTT CATGGCCTAT GAGCAAGTA AATATCGTGC AGTCACTCAG TGTATATCCT TTAACACAGCA GTTGTGTGAT TGTTCCTGG apyI(dcm+)
 TTTAAATTA AATTGGAAA GTACCGGATA CTCGTTTCAT TTATAGCAGC TCAGTGAGTC ACGAATAGGA AATTGTCTGT CAACACACTA ACAAGGACC
 726 N F N L T F S W P M S K V N I V Q S L S A Y P L N S S C V I V S W
 bslI
 haeIII/palI
 ddeI
 draIII
 maeIII
 ahaIII/draI
 tru9I
 mseI
 xmnI
 mvaI
 ecorII
 dsav
 bstNI
 2401 ATACTATCAC CCAGTGATTA CAGCTAATG TATTTATTA TTGAGTGGAA AAATCTTAAT GAAGATGGTG AAATAAAATG GCTTAGAATC TCTTCATCTG earI/ksp632I
 TATGATAGTG GGTCACTAAT GTTCGATTAC ATAAATAAT AACTCACCTT TTAGAATTA CTTCTACCAC TTATTTTAC CGAATCTTAG AGAAGTAGAC
 759 I L S P S D Y K L M Y F I I E W K N L N E D G E I K W L R I S S S V
 draIII
 hphI bsrI
 aluI
 tru9I
 mseI
 mboII hphI
 asp700
 xmnI
 tfII
 hinfI mboII

FIG. 1F

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sau3AI
mboI/ndeII(dam-)
dpsI(dam+)
dpsII(dam-)
    bsrI
    rsaI
    nlaIII
2501 TTAAGAAGTA TTATATCCAT GATCATTTTA TCCTCCATTGA GAAGTACCCAG TTCAGTCTTT ACCCAATATT TATGGAAGGA GTGGGAAGAAC CAAAGATAAT
    mseI
    asel/asnI/vspI
793 K K Y Y I H D H F I P I E K Y Q F S L Y P I F M E G V G K P K I I
    bsrI
    rsaI
    csp6I
    ssfI
2601 TAATAGTTTC ACTCAAGATG ATATTGAAAA ACACCAGAGT GATGCAGGTT TATATGTAAT TGTGCCAGTA ATTATTTCCT CTTCCATCTT ATTGCTTGGA
    sfaNI
    bsrI
    mnlI
826 N S F T Q D D I E K H Q S D A G L Y V I V P V I I S S S I L L G
    bspMI
    sfaNI
    bsrI
    mnlI
    bsp1286
    bmyI
    scrFI
    mvaI
    ecorII
    dsav
    bstNI
    bsaJI
    tru9I
    mseI
    apyI(dcm+)
    CCTGGGCACA AGGACTTAAT TTTCAGAAGC
    859 T L L I S H Q R M K K L F W E D V P N P K N C S W A Q G L N F Q K P
    xmnI
    mboII
    asp700
    aluI
    8701 ACATTATTAA TATCACACCA AAGAATGAAA AGCTATTTT GCGAAGATGT TCCGAACCCC AAGAATTGTT TTTGGAGCC TGAACAATT TCAGAGATA TCAGTGTGA
    TGTAAATATT ATAGTGTGGT TTCTTACTTT TTCGATAAAA CCTTCTACA AGCTTGGG TTCTTAACA GACCCGTGT TCCTGAATTA AAGTCTTCG
    859 T L L I S H Q R M K K L F W E D V P N P K N C S W A Q G L N F Q K P
    nlaIII
    nsPI
    nsPHI
    aflIII
    eam1105I
    mnlI
    asuI
    mboII
    nlaIV
    8801 CAGAAACGTT TGACATCTT TTATCAAGC ATACAGCATC AGTGACATGT GGTCTCTTTC TTTGGAGCC TGAACAATT TCAGAGATA TCAGTGTGA
    GTCTTTGCAA ACTCGTAGAA AATAGTTTCG TATGTCGTAG TCACTGTACA CCAGGAGAG AAGACCTCGG ACTTGTAA AGTCTTCTAT AGTCACAAC
    893 E T F E H L F I K H T A S V T C G P L L L E P E T I S E D I S V D
    maeII
    pep1406I
    sfaNI
    ecorV
    mboII

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FIG. 1G

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sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
bstYI/xhoII
bsrI
maeIII
2901 TACATCATGG AAAAATAAAG ATGAGATGAT GCCAACAACT GTGGTCTCTC TACTTTCAC AACAGATCTT GAAAAGGTT CTGTTTGTAT TAGTGACCAG
ATGATGATACC TTTTATTTC TACTCTACTA CGGTGTGTTGA CACCAGAGAG ATGAAAGTTG TTGTCTAGAA CTTTCCCAA GACAAACATA ATCACTGGTC
926 T S W K N K D E M M P T T V V S L L S T T D L E K G S V C I S D Q

bsmAI
bsaI
sfaNI
2901 TACATCATGG AAAAATAAAG ATGAGATGAT GCCAACAACT GTGGTCTCTC TACTTTCAC AACAGATCTT GAAAAGGTT CTGTTTGTAT TAGTGACCAG
ATGATGATACC TTTTATTTC TACTCTACTA CGGTGTGTTGA CACCAGAGAG ATGAAAGTTG TTGTCTAGAA CTTTCCCAA GACAAACATA ATCACTGGTC
926 T S W K N K D E M M P T T V V S L L S T T D L E K G S V C I S D Q

bsmAI
bsaI
sfaNI
3001 TTCAACAGTG TTAACCTTCTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTGTGTAA ATACGCCACG CTGATCAGCA
AAGTGTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGGATACT CCTGCTTCG GTCTCTCTG GGAACAATT TATCGGTGC GACTAGTCGT
959 F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N

tru9I
mseI
hpaI
hincII/hindII mnlI mnlI csp6I bsteII mnlI
3001 TTCAACAGTG TTAACCTTCTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTGTGTAA ATACGCCACG CTGATCAGCA
AAGTGTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGGATACT CCTGCTTCG GTCTCTCTG GGAACAATT TATCGGTGC GACTAGTCGT
959 F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N

draII
hphI
hphI
hphI
3101 ACTCTAAACC AAGTGAACCT GGTGAAGAAC AAGGGCTTAT AAATAGTTCA GTCACCAAGT GCTTCTCTAG CAAAATTTCT CCGTTGAAGG ATTCTTTCTC
TGAGATTGG TTCACTTTGA CCACCTTCTG CCACCTTCTG TTCCCGAATA TTTATCAAGT CAGTGTGTTCA CGAAGAGATC GTTTTAAAG GGCACACTCC TAAGAAAGAG
993 S K P S E T G E E Q G L I N S S V T K C F S S K N S P L K D S F S

scrFI
mvaI
ecorII
dsav
bstNI
apyI[dcmt+]
sau96I
haeIII/palI
asui
nlaIII
aluI
3201 TAATAGTCA TGGAGATAG AGGCCAGGC ATTTTATATA TTATCAGATC AGCATCCCAA CATAATTCA CCACACCTCA CATTCTCAGA AGGATTGGAT
ATTATCGAGT ACCCTTATC TCCGGTCCG TAAATAATAT AATAGTCTAG TCGTAGGTT GTATTAAGT GGTGTGGAGT GTAAGAGTCT TCCTAACCTA
1026 N S S W E I E A Q A F F I L S D Q H P N I I S P H L T F S E G L D

sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
bstYI/xhoII
bsrI
maeIII
2901 TACATCATGG AAAAATAAAG ATGAGATGAT GCCAACAACT GTGGTCTCTC TACTTTCAC AACAGATCTT GAAAAGGTT CTGTTTGTAT TAGTGACCAG
ATGATGATACC TTTTATTTC TACTCTACTA CGGTGTGTTGA CACCAGAGAG ATGAAAGTTG TTGTCTAGAA CTTTCCCAA GACAAACATA ATCACTGGTC
926 T S W K N K D E M M P T T V V S L L S T T D L E K G S V C I S D Q

bsmAI
bsaI
sfaNI
2901 TACATCATGG AAAAATAAAG ATGAGATGAT GCCAACAACT GTGGTCTCTC TACTTTCAC AACAGATCTT GAAAAGGTT CTGTTTGTAT TAGTGACCAG
ATGATGATACC TTTTATTTC TACTCTACTA CGGTGTGTTGA CACCAGAGAG ATGAAAGTTG TTGTCTAGAA CTTTCCCAA GACAAACATA ATCACTGGTC
926 T S W K N K D E M M P T T V V S L L S T T D L E K G S V C I S D Q

bsmAI
bsaI
sfaNI
3001 TTCAACAGTG TTAACCTTCTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTGTGTAA ATACGCCACG CTGATCAGCA
AAGTGTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGGATACT CCTGCTTCG GTCTCTCTG GGAACAATT TATCGGTGC GACTAGTCGT
959 F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N

tru9I
mseI
hpaI
hincII/hindII mnlI mnlI csp6I bsteII mnlI
3001 TTCAACAGTG TTAACCTTCTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTGTGTAA ATACGCCACG CTGATCAGCA
AAGTGTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGGATACT CCTGCTTCG GTCTCTCTG GGAACAATT TATCGGTGC GACTAGTCGT
959 F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N

draII
hphI
hphI
hphI
3101 ACTCTAAACC AAGTGAACCT GGTGAAGAAC AAGGGCTTAT AAATAGTTCA GTCACCAAGT GCTTCTCTAG CAAAATTTCT CCGTTGAAGG ATTCTTTCTC
TGAGATTGG TTCACTTTGA CCACCTTCTG CCACCTTCTG TTCCCGAATA TTTATCAAGT CAGTGTGTTCA CGAAGAGATC GTTTTAAAG GGCACACTCC TAAGAAAGAG
993 S K P S E T G E E Q G L I N S S V T K C F S S K N S P L K D S F S

scrFI
mvaI
ecorII
dsav
bstNI
apyI[dcmt+]
sau96I
haeIII/palI
asui
nlaIII
aluI
3201 TAATAGTCA TGGAGATAG AGGCCAGGC ATTTTATATA TTATCAGATC AGCATCCCAA CATAATTCA CCACACCTCA CATTCTCAGA AGGATTGGAT
ATTATCGAGT ACCCTTATC TCCGGTCCG TAAATAATAT AATAGTCTAG TCGTAGGTT GTATTAAGT GGTGTGGAGT GTAAGAGTCT TCCTAACCTA
1026 N S S W E I E A Q A F F I L S D Q H P N I I S P H L T F S E G L D

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FIG. 1H

FIG. 11

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sau96I      sau96I
nlaIV       nlaIV
avaII       avaII
asul        asul
rmaI        rmaI
mael        mael
ppuMI       ppuMI
ecol109I/draII  aluI
TCCAGCTAGA AATAAGCCCA ACAGACACCA
AGTATTCGG ATGTTACAT CTGTGCGAGA AGATAAATA AAGGTTTCGAG ATCACCCTTC CAGGGAACAA AGGTCGATCT TTATTCGGGT TGTCTGTGGT

3801 TCCATAAGCC TACCAATGTA GACACGCTCT TCTATTTTAT TCCCAAGCTC TAGTGGGAAG GTCCCTTGT TCCAGCTAGA AATAAGCCCA ACAGACACCA
AAGTATTCGG ATGTTACAT CTGTGCGAGA AGATAAATA AAGGTTTCGAG ATCACCCTTC CAGGGAACAA AGGTCGATCT TTATTCGGGT TGTCTGTGGT

mboII      earI/ksp632I      sapi      accI
TCCATAAGCC TACCAATGTA GACACGCTCT TCTATTTTAT TCCCAAGCTC TAGTGGGAAG GTCCCTTGT TCCAGCTAGA AATAAGCCCA ACAGACACCA
AAGTATTCGG ATGTTACAT CTGTGCGAGA AGATAAATA AAGGTTTCGAG ATCACCCTTC CAGGGAACAA AGGTCGATCT TTATTCGGGT TGTCTGTGGT

3901 TCTTTTGTGA GATGTAATTG TTTTTCGAGA GGGCGTGTG TTTTACCTCA AGTTTGTGT TGTATCCCAAC ACACACACAC ACACACATTC TTAACACATG
AGAAACACT CTACATTAAC AAAAAGTCT CCGGCACAC CCAAGTGTG TTTTACCTCA AGTTTGTGT TGTATCCCAAC ACACACACAC ACACACATTC TTAACACATG
TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG

nspi      nspi
tru9I nlaII
msei aflIII
TAAACACATG
AATTGTGTAC

sfuI      bstBI      bsiCI      asuII
tru9I      ecoRI      apoI
msei
TAAACAGAAAT
TACAATTTCT ATTGTCTTCA

4001 TCCTTGTGTG TTTTGAGAGT ATATTATGTA TTTATATTTT GTGCTATCAG ACTGTAGGAT TTGAAGTAGG ACTTTCCTAA ATGTTTAAAG TAAACAGAAAT
AGGAACACAC AAAACTCTCA TATAATACAT AAATATAAAA CACGATAGTC TGACATCCTA AACTTCATCC TGAAGGATT TACAATTTCT ATTGTCTTCA

taqI
4101 TC
AG

length: 4102

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FIG. 1J

wsxfull.6.4.variant	1	M I C Q K F C V V L L H W E F I Y V I T A F N L S Y P I T P W R F K L S C M P P N S T Y D Y F L L P
wsxfull.12.1.variant	1	M I C Q K F C V V L L H W E F I Y V I T A F N L S Y P I T P W R F K L S C M P P N S T Y D Y F L L P
wsxfull.13.2.variant	1	M I C Q K F C V V L L H W E F I Y V I T A F N L S Y P I T P W R F K L S C M P P N S T Y D Y F L L P
wsxfull.6.4.variant	51	A G L S K N T S N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R S E O D R N C S
wsxfull.12.1.variant	51	A G L S K N T S N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R S E O D R N C S
wsxfull.13.2.variant	51	A G L S K N T S N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R S E O D R N C S
wsxfull.6.4.variant	101	L C A D N I E G K T F V S T V N S L V F Q O I D A N W N I O C W L K G D L K L F I C Y V E S L F K N
wsxfull.12.1.variant	101	L C A D N I E G K T F V S T V N S L V F Q O I D A N W N I O C W L K G D L K L F I C Y V E S L F K N
wsxfull.13.2.variant	101	L C A D N I E G K T F V S T V N S L V F Q O I D A N W N I O C W L K G D L K L F I C Y V E S L F K N
wsxfull.6.4.variant	151	L F R N Y N Y K V H L L Y V L P E V L E D S P L V P O K G S F O M V H C N C S V H E C C E C L V P V
wsxfull.12.1.variant	151	L F R N Y N Y K V H L L Y V L P E V L E D S P L V P O K G S F O M V H C N C S V H E C C E C L V P V
wsxfull.13.2.variant	151	L F R N Y N Y K V H L L Y V L P E V L E D S P L V P O K G S F O M V H C N C S V H E C C E C L V P V
wsxfull.6.4.variant	201	P T A K L N D T L L M C L K I T S G G V I F O S P L M S V O P I N M V K P D P P L G L H M E I T D D
wsxfull.12.1.variant	201	P T A K L N D T L L M C L K I T S G G V I F O S P L M S V O P I N M V K P D P P L G L H M E I T D D
wsxfull.13.2.variant	201	P T A K L N D T L L M C L K I T S G G V I F O S P L M S V O P I N M V K P D P P L G L H M E I T D D
wsxfull.6.4.variant	251	G N L K I S W S S P P L V P F P L Q Y O V K Y S E N S T T V I R E A D K I V S A T S L L V D S I L P
wsxfull.12.1.variant	251	G N L K I S W S S P P L V P F P L Q Y O V K Y S E N S T T V I R E A D K I V S A T S L L V D S I L P
wsxfull.13.2.variant	251	G N L K I S W S S P P L V P F P L Q Y O V K Y S E N S T T V I R E A D K I V S A T S L L V D S I L P

FIG. 2A

wsxfull.6.4.variant	301	GSSYEVRGKRLDGGIWSDWSTPRVFTTQDVIFYFPPKILTSVGSNVVSF
wsxfull.12.1.variant	301	GSSYEVRGKRLDGGIWSDWSTPRVFTTQDVIFYFPPKILTSVGSNVVSF
wsxfull.13.2.variant	301	GSSYEVRGKRLDGGIWSDWSTPRVFTTQDVIFYFPPKILTSVGSNVVSF
wsxfull.6.4.variant	351	HCIYKKENKIVPSKEIVWWMNLAEKIPQSOYDVSDHVSKVTFNLFNETK
wsxfull.12.1.variant	351	HCIYKKENKIVPSKEIVWWMNLAEKIPQSOYDVSDHVSKVTFNLFNETK
wsxfull.13.2.variant	351	HCIYKKENKIVPSKEIVWWMNLAEKIPQSOYDVSDHVSKVTFNLFNETK
wsxfull.6.4.variant	401	PRGKFTYDAVYCCNEHECHRYAELYVIDVNIINISCE TDGYLT KMT CRWS
wsxfull.12.1.variant	401	PRGKFTYDAVYCCNEHECHRYAELYVIDVNIINISCE TDGYLT KMT CRWS
wsxfull.13.2.variant	401	PRGKFTYDAVYCCNEHECHRYAELYVIDVNIINISCE TDGYLT KMT CRWS
wsxfull.6.4.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHP ISEPKDCYLOSDGFYECIF
wsxfull.12.1.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHP ISEPKDCYLOSDGFYECIF
wsxfull.13.2.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHP ISEPKDCYLOSDGFYECIF
wsxfull.6.4.variant	501	OPIFLSGYTMWIRINHSLSGLDSPPTCVLPDSVVKPLPPSSVKAETIN
wsxfull.12.1.variant	501	OPIFLSGYTMWIRINHSLSGLDSPPTCVLPDSVVKPLPPSSVKAETIN
wsxfull.13.2.variant	501	OPIFLSGYTMWIRINHSLSGLDSPPTCVLPDSVVKPLPPSSVKAETIN
wsxfull.6.4.variant	551	IGLLKISWEKPVFPENNLOFOIRYGLSGKEVQWKMYEYDAKSKSVSLPV
wsxfull.12.1.variant	551	IGLLKISWEKPVFPENNLOFOIRYGLSGKEVQWKMYEYDAKSKSVSLPV
wsxfull.13.2.variant	551	IGLLKISWEKPVFPENNLOFOIRYGLSGKEVQWKMYEYDAKSKSVSLPV

FIG. 2B

wsxfull.6.4.variant	601	PDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDIKVPMRGPEFWRIIN
wsxfull.12.1.variant	601	PDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDIKVPMRGPEFWRIIN
wsxfull.13.2.variant	601	PDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDIKVPMRGPEFWRIIN
wsxfull.6.4.variant	651	GDTMKKEKNVTLLWKPLMKNDSLCSVORYVINHHHTSCNGT WSE DVG NHTK
wsxfull.12.1.variant	651	GDTMKKEKNVTLLWKPLMKNDSLCSVORYVINHHHTSCNGT WSE DVG NHTK
wsxfull.13.2.variant	651	GDTMKKEKNVTLLWKPLMKNDSLCSVORYVINHHHTSCNGT WSE DVG NHTK
wsxfull.6.4.variant	701	FTFLWTEOAHTVTVLAINSIGASVANFNLTFSWPMSKVNIVOSLSAYPLN
wsxfull.12.1.variant	701	FTFLWTEOAHTVTVLAINSIGASVANFNLTFSWPMSKVNIVOSLSAYPLN
wsxfull.13.2.variant	701	FTFLWTEOAHTVTVLAINSIGASVANFNLTFSWPMSKVNIVOSLSAYPLN
wsxfull.6.4.variant	751	SSCVIVSWILSPSDYKLMYFIEWKNLNEDGEIKWLRISSSVKKYIHDH
wsxfull.12.1.variant	751	SSCVIVSWILSPSDYKLMYFIEWKNLNEDGEIKWLRISSSVKKYIHDH
wsxfull.13.2.variant	751	SSCVIVSWILSPSDYKLMYFIEWKNLNEDGEIKWLRISSSVKKYIHDH
wsxfull.6.4.variant	801	FIPIEKYQFSLYPIFMEGVGKPKIINSFTODDIEKHQSDAGLYVIVPVI
wsxfull.12.1.variant	801	FIPIEKYQFSLYPIFMEGVGKPKIINSFTODDIEKHQSDAGLYVIVPVI
wsxfull.13.2.variant	801	FIPIEKYQFSLYPIFMEGVGKPKIINSFTODDIEKHQSDAGLYVIVPVI
wsxfull.6.4.variant	851	membrane Domain
wsxfull.12.1.variant	851	SSSILLGLTLLISHOR
wsxfull.13.2.variant	851	SSSILLGLTLLISHOR
wsxfull.13.2.variant	851	SSSILLGLTLLISHOR
		Box 1
		KKLFWEDVPNPKNCSWAQGLNFOK
		KKLFWEDVPNPKNCSWAQGLNFOK
		KKLFWEDVPNPKNCSWAQGLNFOK
		MF
		PETFEHLFI

FIG. 2C

wsxfull.13.2.variant 901 KHTASVTC[G]PLLEPETISEDISVDTSWKNKDEMMPTTVVSLSTTDLEK Box 2
 wsxfull.13.2.variant 951 GSVCSIS]DOF[NSVNFSEAEGETEYEDESQRQ]PFFVKYATLISNSKPSETGE Box 3
 wsxfull.6.4.variant 892
 wsxfull.12.1.variant 894
 wsxfull.13.2.variant 1001 EOGLINSSVTKCFSSKN SPLKOSFSN SWEIEAQAFFILSDQH[P]N[I]SPH
 wsxfull.6.4.variant 893
 wsxfull.12.1.variant 903
 wsxfull.13.2.variant 1051 LTFSEGLDELLKLEGNFPEENNDKKS IYYLGVTSIKKRESGV[L]LTOKSRV
 wsxfull.12.1.variant 908 R[C]L[K]A[A]C[S]L[R]V[I]TTP.....
 wsxfull.13.2.variant 1101 SCPFPAPCLFTD[R]V[L]QDS[C]S[H]F[V]E[N]N I N L G T S S K K T F A S Y M P O F O T C S T
 wsxfull.13.2.variant 1151 QTHKIMENKMCDLTY

FIG. 2D

wsxfull.6.4.variant	1	G A A T T C C G G G T T A A A G C T C T C G T G G C A T T A T C C T T C A G T G G G G C T A T T G G
wsxfull.6.4.variant	51	A C T G A C T T T T C T T A T G C T G G G A T G T G C C T T A G A G G A T T A T G G A T T T G C C A
wsxfull.12.1.variant	1
wsxfull.13.2.variant	1
wsxfull.6.4.variant	101	G T T C A C C C T G A C C A T C T T G A A A A T A A G T T A T C T C T G A T C T G T C T G T A T
wsxfull.12.1.variant	14	G A C G G C G G G C G T T A A A G C T C T C G T G G C A T T A T C C T T C A G T G G G G C T A T T G
wsxfull.13.2.variant	14	G A C G G C G G G C G T T A A A G C T C T C G T G G C A T T A T C C T T C A G T G G G G C T A T T G
wsxfull.6.4.variant	151	G T T A C T T C T C T C C C T C A C C A A T G G A G A A C A A T G T G G G C A A A G T G T A C T
wsxfull.12.1.variant	64	G A C T G A C T T T T C T T A T G C T G G G A T G T G C C T T A G A G G A T T A T G G G T G T A C T
wsxfull.13.2.variant	64	G A C T G A C T T T T C T T A T G C T G G G A T G T G C C T T A G A G G A T T A T G G G T G T A C T
wsxfull.6.4.variant	201	T C T C T G A A G T A A G A T G A T T T G T C A A A A A T T C T G T G T G G T T T T G T T A C A T T
wsxfull.12.1.variant	114	T C T C T G A A G T A A G A T G A T T T G T C A A A A A T T C T G T G T G G T T T T G T T A C A T T
wsxfull.13.2.variant	114	T C T C T G A A G T A A G A T G A T T T G T C A A A A A T T C T G T G T G G T T T T G T T A C A T T
wsxfull.6.4.variant	251	G G G A A T T T A T T T A T G T G A T A A C T G C G T T T A A C T T G T C A T A T C C A A T T A C T
wsxfull.12.1.variant	164	G G G A A T T T A T T T A T G T G A T A A C T G C G T T T A A C T T G T C A T A T C C A A T T A C T
wsxfull.13.2.variant	164	G G G A A T T T A T T T A T G T G A T A A C T G C G T T T A A C T T G T C A T A T C C A A T T A C T
wsxfull.6.4.variant	301	C C T T G G A G A T T T A A G T T G T C T T G C A T G C C A C C A A A T T C A A C C T A T G A C T A
wsxfull.12.1.variant	214	C C T T G G A G A T T T A A G T T G T C T T G C A T G C C A C C A A A T T C A A C C T A T G A C T A
wsxfull.13.2.variant	214	C C T T G G A G A T T T A A G T T G T C T T G C A T G C C A C C A A A T T C A A C C T A T G A C T A

FIG. 3A

wsxfull.6.4.variant	351	C T T C C T T T T G C C T G C T G G A C T C T C A A G A A T A C T T C A A A T T C G A A T G G A C
wsxfull.12.1.variant	264	C T T C C T T T T G C C T G C T G G A C T C T C A A G A A T A C T T C A A A T T C G A A T G G A C
wsxfull.13.2.variant	264	C T T C C T T T T G C C T G C T G G A C T C T C A A G A A T A C T T C A A A T T C G A A T G G A C
wsxfull.6.4.variant	401	A T T A T G A G A C A G C T G T T G A A C C T A A G T T T A A T T C A A G T G G T A C T C A C T T T
wsxfull.12.1.variant	314	A T T A T G A G A C A G C T G T T G A A C C T A A G T T T A A T T C A A G T G G T A C T C A C T T T
wsxfull.13.2.variant	314	A T T A T G A G A C A G C T G T T G A A C C T A A G T T T A A T T C A A G T G G T A C T C A C T T T
wsxfull.6.4.variant	451	T C T A A C T T A T C C A A A A C A A C T T T C C A C T G T T G C T T T C G G A G T G A G C A A G A
wsxfull.12.1.variant	364	T C T A A C T T A T C C A A A A C A A C T T T C C A C T G T T G C T T T C G G A G T G A G C A A G A
wsxfull.13.2.variant	364	T C T A A C T T A T C C A A A A C A A C T T T C C A C T G T T G C T T T C G G A G T G A G C A A G A
wsxfull.6.4.variant	501	T A G A A A C T G C T C C T T A T G T G C A G A C A A C A T T G A A G G A A A G A C A T T T G T T T
wsxfull.12.1.variant	414	T A G A A A C T G C T C C T T A T G T G C A G A C A A C A T T G A A G G A A A G A C A T T T G T T T
wsxfull.13.2.variant	414	T A G A A A C T G C T C C T T A T G T G C A G A C A A C A T T G A A G G A A A G A C A T T T G T T T
wsxfull.6.4.variant	551	C N A C A G T A A A T T C T T T A G T T T T C A A C A A A T A G A T G C A A A C T G G A A C A T A
wsxfull.12.1.variant	464	C A A C A G T A A A T T C T T T A G T T T T C A A C A A A T A G A T G C A A A C T G G A A C A T A
wsxfull.13.2.variant	464	C A A C A G T A A A T T C T T T A G T T T T C A A C A A A T A G A T G C A A A C T G G A A C A T A
wsxfull.6.4.variant	601	C A G T G C T G G C T A A A A G G A G A C T T A A A A T T A T T C A T C T G T T A T G T G G A G T C
wsxfull.12.1.variant	514	C A G T G C T G G C T A A A A G G A G A C T T A A A A T T A T T C A T C T G T T A T G T G G A G T C
wsxfull.13.2.variant	514	C A G T G C T G G C T A A A A G G A G A C T T A A A A T T A T T C A T C T G T T A T G T G G A G T C

FIG. 3B

wsxfull.6.4.variant	651	A T T A T T T A A G A A T C T A T T C A G G A A T T A A C T A T A A G G T C C A T C T T T T A T
wsxfull.12.1.variant	564	A T T A T T T A A G A A T C T A T T C A G G A A T T A A C T A T A A G G T C C A T C T T T T A T
wsxfull.13.2.variant	564	A T T A T T T A A G A A T C T A T T C A G G A A T T A A C T A T A A G G T C C A T C T T T T A T
wsxfull.6.4.variant	701	A T G T T C T G C C T G A A G T G T T A G A A G A T T C A C C T C T G G T T C C C C A A A A A G G C
wsxfull.12.1.variant	614	A T G T T C T G C C T G A A G T G T T A G A A G A T T C A C C T C T G G T T C C C C A A A A A G G C
wsxfull.13.2.variant	614	A T G T T C T G C C T G A A G T G T T A G A A G A T T C A C C T C T G G T T C C C C A A A A A G G C
wsxfull.6.4.variant	751	A G T T T T C A G A T G G T T C A C T G C A A T T G C A G T G T T C A T G A A T G T T G T G A A T G
wsxfull.12.1.variant	664	A G T T T T C A G A T G G T T C A C T G C A A T T G C A G T G T T C A T G A A T G T T G T G A A T G
wsxfull.13.2.variant	664	A G T T T T C A G A T G G T T C A C T G C A A T T G C A G T G T T C A T G A A T G T T G T G A A T G
wsxfull.6.4.variant	801	T C T T G T G C C T G T G C C A A C A G C C A A A C T C A A C G A C A C T C T C C T T A T G T G T T
wsxfull.12.1.variant	714	T C T T G T G C C T G T G C C A A C A G C C A A A C T C A A C G A C A C T C T C C T T A T G T G T T
wsxfull.13.2.variant	714	T C T T G T G C C T G T G C C A A C A G C C A A A C T C A A C G A C A C T C T C C T T A T G T G T T
wsxfull.6.4.variant	851	T G A A A A T C A C A T C T G G T G G A G T A A T T T C C A G T C A C C T C T A A T G T C A G T T
wsxfull.12.1.variant	764	T G A A A A T C A C A T C T G G T G G A G T A A T T T C C A G T C A C C T C T A A T G T C A G T T
wsxfull.13.2.variant	764	T G A A A A T C A C A T C T G G T G G A G T A A T T T C C A G T C A C C T C T A A T G T C A G T T
wsxfull.6.4.variant	901	C A G C C C A T A A A T A T G G T G A A G C C T G A T C C A C C A T T A G G T T T G C A T A T G G A
wsxfull.12.1.variant	814	C A G C C C A T A A A T A T G G T G A A G C C T G A T C C A C C A T T A G G T T T G C A T A T G G A
wsxfull.13.2.variant	814	C A G C C C A T A A A T A T G G T G A A G C C T G A T C C A C C A T T A G G T T T G C A T A T G G A

FIG. 3C

wsxfull.6.4.variant	951	AATCACAAGATGATGGTAATTATAAGATTTCTTGGTCCAGCCACCAATTGG
wsxfull.12.1.variant	964	AATCACAAGATGATGGTAATTATAAGATTTCTTGGTCCAGCCACCAATTGG
wsxfull.13.2.variant	964	AATCACAAGATGATGGTAATTATAAGATTTCTTGGTCCAGCCACCAATTGG
wsxfull.6.4.variant	1001	TACCAATTTCCACTTCAATATCAAGTGAAATATTTCAGAGAATTCTACAACA
wsxfull.12.1.variant	914	TACCAATTTCCACTTCAATATCAAGTGAAATATTTCAGAGAATTCTACAACA
wsxfull.13.2.variant	914	TACCAATTTCCACTTCAATATCAAGTGAAATATTTCAGAGAATTCTACAACA
wsxfull.6.4.variant	1051	GTTATCAGAGAAGCTGACAGAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.12.1.variant	964	GTTATCAGAGAAGCTGACAGAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.13.2.variant	964	GTTATCAGAGAAGCTGACAGAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.6.4.variant	1101	CAGTATACTTCCCTGGGTCTTCGTATGAGGTTCAAGGTGAGGGGCAAGAGAC
wsxfull.12.1.variant	1014	CAGTATACTTCCCTGGGTCTTCGTATGAGGTTCAAGGTGAGGGGCAAGAGAC
wsxfull.13.2.variant	1014	CAGTATACTTCCCTGGGTCTTCGTATGAGGTTCAAGGTGAGGGGCAAGAGAC
wsxfull.6.4.variant	1151	TGGATGGCCCCAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.12.1.variant	1064	TGGATGGCCCCAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.13.2.variant	1064	TGGATGGCCCCAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.6.4.variant	1201	ACACAAGATGTCATATACCTTCCACCTAAATAATTCTGACAAGTGTGGGTCT
wsxfull.12.1.variant	1114	ACACAAGATGTCATATACCTTCCACCTAAATAATTCTGACAAGTGTGGGTCT
wsxfull.13.2.variant	1114	ACACAAGATGTCATATACCTTCCACCTAAATAATTCTGACAAGTGTGGGTCT

FIG. 3D

wsxfull.6.4.variant	1251	T A A T G T T T C T T T T C A C T G C A T C T A T A A G A A G G A A A C A A G A T T G T T C C C T
wsxfull.12.1.variant	1164	T A A T G T T T C T T T T C A C T G C A T C T A T A A G A A G G A A A C A A G A T T G T T C C C T
wsxfull.13.2.variant	1164	T A A T G T T T C T T T T C A C T G C A T C T A T A A G A A G G A A A C A A G A T T G T T C C C T
wsxfull.6.4.variant	1301	C A A A G A G A T T G T T T G G T G G A T G A A T T A G C T G A G A A A A T T C C T C A A A G C
wsxfull.12.1.variant	1214	C A A A G A G A T T G T T T G G T G G A T G A A T T A G C T G A G A A A A T T C C T C A A A G C
wsxfull.13.2.variant	1214	C A A A G A G A T T G T T T G G T G G A T G A A T T A G C T G A G A A A A T T C C T C A A A G C
wsxfull.6.4.variant	1351	C A G T A T G A T G T T G T G A G T G A T C A T G T T A G C A A A G T T A C T T T T T C A A T C T
wsxfull.12.1.variant	1264	C A G T A T G A T G T T G T G A G T G A T C A T G T T A G C A A A G T T A C T T T T T C A A T C T
wsxfull.13.2.variant	1264	C A G T A T G A T G T T G T G A G T G A T C A T G T T A G C A A A G T T A C T T T T T C A A T C T
wsxfull.6.4.variant	1401	G A A T G A A A C C A A A C C T C G A G G A A A G T T T A C C T A T G A T G C A G T G T A C T G C T
wsxfull.12.1.variant	1314	G A A T G A A A C C A A A C C T C G A G G A A A G T T T A C C T A T G A T G C A G T G T A C T G C T
wsxfull.13.2.variant	1314	G A A T G A A A C C A A A C C T C G A G G A A A G T T T A C C T A T G A T G C A G T G T A C T G C T
wsxfull.6.4.variant	1451	G C A A T G A A C A T G A A T G C C A T C A T C G C T A T G C T G A A T T A T A T G T G A T T G A T
wsxfull.12.1.variant	1364	G C A A T G A A C A T G A A T G C C A T C A T C G C T A T G C T G A A T T A T A T G T G A T T G A T
wsxfull.13.2.variant	1364	G C A A T G A A C A T G A A T G C C A T C A T C G C T A T G C T G A A T T A T A T G T G A T T G A T
wsxfull.6.4.variant	1501	G T C A A T A T C A A T A T C T C A T G T G A A A C T G A T G G G T A C T T A A C T A A A A T G A C
wsxfull.12.1.variant	1414	G T C A A T A T C A A T A T C T C A T G T G A A A C T G A T G G G T A C T T A A C T A A A A T G A C
wsxfull.13.2.variant	1414	G T C A A T A T C A A T A T C T C A T G T G A A A C T G A T G G G T A C T T A A C T A A A A T G A C

FIG. 3E

wsxfull.6.4.variant	1551	T T G C A G A T G G T C A A C C A G T A C A A T C C A G T C A C T T G C G G A A A G C A C T T T G C
wsxfull.12.1.variant	1464	T T G C A G A T G G T C A A C C A G T A C A A T C C A G T C A C T T G C G G A A A G C A C T T T G C
wsxfull.13.2.variant	1464	T T G C A G A T G G T C A A C C A G T A C A A T C C A G T C A C T T G C G G A A A G C A C T T T G C
wsxfull.6.4.variant	1601	A A T T G A G G T A T C A T A G G A G C A G C C T T T A C T G T T C T G A T A T T C C A T C T A T T
wsxfull.12.1.variant	1514	A A T T G A G G T A T C A T A G G A G C A G C C T T T A C T G T T C T G A T A T T C C A T C T A T T
wsxfull.13.2.variant	1514	A A T T G A G G T A T C A T A G G A G C A G C C T T T A C T G T T C T G A T A T T C C A T C T A T T
wsxfull.6.4.variant	1631	C A T C C C A T A T C T G A G C C C A A A G A T T G C T A T T T G C A G A G T G A T G G T T T T A
wsxfull.12.1.variant	1564	C A T C C C A T A T C T G A G C C C A A A G A T T G C T A T T T G C A G A G T G A T G G T T T T A
wsxfull.13.2.variant	1564	C A T C C C A T A T C T G A G C C C A A A G A T T G C T A T T T G C A G A G T G A T G G T T T T A
wsxfull.6.4.variant	1701	T G A A T G C A T T T T C C A G C C A A T C T T C C T A T T A T C T G G C T A C A C A A T G T G G A
wsxfull.12.1.variant	1614	T G A A T G C A T T T T C C A G C C A A T C T T C C T A T T A T C T G G C T A C A C A A T G T G G A
wsxfull.13.2.variant	1614	T G A A T G C A T T T T C C A G C C A A T C T T C C T A T T A T C T G G C T A C A C A A T G T G G A
wsxfull.6.4.variant	1751	T T A G G A T C A A T C A C T C T C T A G G T T C A C T T G A C T C T C C A C C A A C A T G T G T C
wsxfull.12.1.variant	1664	T T A G G A T C A A T C A C T C T C T A G G T T C A C T T G A C T C T C C A C C A A C A T G T G T C
wsxfull.13.2.variant	1664	T T A G G A T C A A T C A C T C T C T A G G T T C A C T T G A C T C T C C A C C A A C A T G T G T C
wsxfull.6.4.variant	1801	C T T C C T G A T T C T G T G G T G A A G C C A C T G C C T C C A T C C A G T G T G A A A G C A G A
wsxfull.12.1.variant	1714	C T T C C T G A T T C T G T G G T G A A G C C A C T G C C T C C A T C C A G T G T G A A A G C A G A
wsxfull.13.2.variant	1714	C T T C C T G A T T C T G T G G T G A A G C C A C T G C C T C C A T C C A G T G T G A A A G C A G A

FIG. 3F

wsxfull.6.4.variant	1831	AATTACTATAAACATTGGATTATTGAAATAATCTTGGAAAGCCAGTCT
wsxfull.12.1.variant	1764	AATTACTATAAACATTGGATTATTGAAATAATCTTGGAAAGCCAGTCT
wsxfull.13.2.variant	1764	AATTACTATAAACATTGGATTATTGAAATAATCTTGGAAAGCCAGTCT
wsxfull.6.4.variant	1901	TTCCAGAGAAATAACCTTCAATTCCAGATTCGGCTATGGTTAAGTGGAAAA
wsxfull.12.1.variant	1814	TTCCAGAGAAATAACCTTCAATTCCAGATTCGGCTATGGTTAAGTGGAAAA
wsxfull.13.2.variant	1814	TTCCAGAGAAATAACCTTCAATTCCAGATTCGGCTATGGTTAAGTGGAAAA
wsxfull.6.4.variant	1931	GAGTACAAATGGAAGATGTATGAGGTTTATGATGCAAAATCAAAATCTGT
wsxfull.12.1.variant	1864	GAGTACAAATGGAAGATGTATGAGGTTTATGATGCAAAATCAAAATCTGT
wsxfull.13.2.variant	1864	GAGTACAAATGGAAGATGTATGAGGTTTATGATGCAAAATCAAAATCTGT
wsxfull.6.4.variant	2001	CAGTCTCCCAGTTCAGACTTGTGTGCAGTCTATGCTGTTCAAGGTGCGCT
wsxfull.12.1.variant	1914	CAGTCTCCCAGTTCAGACTTGTGTGCAGTCTATGCTGTTCAAGGTGCGCT
wsxfull.13.2.variant	1914	CAGTCTCCCAGTTCAGACTTGTGTGCAGTCTATGCTGTTCAAGGTGCGCT
wsxfull.6.4.variant	2051	GTAAGAGGCTAGATGGACTGGGATATTGGAGTAATTGGAGCAATCCAGCC
wsxfull.12.1.variant	1964	GTAAGAGGCTAGATGGACTGGGATATTGGAGTAATTGGAGCAATCCAGCC
wsxfull.13.2.variant	1964	GTAAGAGGCTAGATGGACTGGGATATTGGAGTAATTGGAGCAATCCAGCC
wsxfull.6.4.variant	2101	TACACAGTTGTCAATGGATATAAAAGTTCCTATGAGAGGACCTGAATTTTG
wsxfull.12.1.variant	2014	TACACAGTTGTCAATGGATATAAAAGTTCCTATGAGAGGACCTGAATTTTG
wsxfull.13.2.variant	2014	TACACAGTTGTCAATGGATATAAAAGTTCCTATGAGAGGACCTGAATTTTG

FIG. 3G

wsxfull.6.4.variant	2151	G A G A T A A T T A A T G G A G A T A C T A T G A A A A A G G A G A A A A T G T C A C T T T A C
wsxfull.12.1.variant	2064	G A G A T A A T T A A T G G A G A T A C T A T G A A A A A G G A G A A A A T G T C A C T T T A C
wsxfull.13.2.variant	2064	G A G A T A A T T A A T G G A G A T A C T A T G A A A A A G G A G A A A A T G T C A C T T T A C
wsxfull.6.4.variant	2201	T T T G G A A G C C C C T G A T G A A A A A T G A C T C A T T G T G C A G T G T T C A G A G A T A T
wsxfull.12.1.variant	2114	T T T G G A A G C C C C C T G A T G A A A A A T G A C T C A T T G T G C A G T G T T C A G A G A T A T
wsxfull.13.2.variant	2114	T T T G G A A G C C C C C T G A T G A A A A A T G A C T C A T T G T G C A G T G T T C A G A G A T A T
wsxfull.6.4.variant	2251	G T G A T A A A C C A T C A T A C T T C C T G C A A T G G A A C A T G G T C A G A A G A T G T G G G
wsxfull.12.1.variant	2164	G T G A T A A A C C A T C A T A C T T C C T G C A A T G G A A C A T G G T C A G A A G A T G T G G G
wsxfull.13.2.variant	2164	G T G A T A A A C C A T C A T A C T T C C T G C A A T G G A A C A T G G T C A G A A G A T G T G G G
wsxfull.6.4.variant	2301	A A A T C A C A C G A A A T T C A C T T T C C T G T G G A C A G A G C A A G C A C A T A C T G T T A
wsxfull.12.1.variant	2214	A A A T C A C A C G A A A T T C A C T T T C C T G T G G A C A C A G A G C A A G C A C A T A C T G T T A
wsxfull.13.2.variant	2214	A A A T C A C A C G A A A T T C A C T T T C C T G T G G A C A C A G A G C A A G C A C A T A C T G T T A
wsxfull.6.4.variant	2351	C G G T T C T G G C C A T C A A T T C A A T T G G T G C T T C T G T T G C A A A T T T T A A T T T A
wsxfull.12.1.variant	2264	C G G T T C T G G C C A T C A A T T C A A T T G G T G C T T C T G T T G C A A A T T T T A A T T T A
wsxfull.13.2.variant	2264	C G G T T C T G G C C A T C A A T T C A A T T G G T G C T T C T G T T G C A A A T T T T A A T T T A
wsxfull.6.4.variant	2401	A C C T T T T C A T G G C C T A T G A G C A A A G T A A A T A T C G T G C A G T C A C T C A G T G C
wsxfull.12.1.variant	2314	A C C T T T T C A T G G C C T A T G A G C A A A G T A A A T A T C G T G C A G T C A C T C A G T G C
wsxfull.13.2.variant	2314	A C C T T T T C A T G G C C T A T G A G C A A A G T A A A T A T C G T G C A G T C A C T C A G T G C

FIG. 3H

wsxfull.6.4.variant	2451	T T A T C C T T T A A A C A G C A G T T G T G A T T G T T T C C T G G A T A C T A T C A C C C A
wsxfull.12.1.variant	2364	T T A T C C T T T A A A C A G C A G T T G T G A T T G T T T C C T G G A T A C T A T C A C C C A
wsxfull.13.2.variant	2364	T T A T C C T T T A A A C A G C A G T T G T G A T T G T T T C C T G G A T A C T A T C A C C C A
wsxfull.6.4.variant	2501	G T G A T T A C A A G C T A A T G T A T T T T A T T A T T G A G T G G A A A A T C T T A A T G A A
wsxfull.12.1.variant	2414	G T G A T T A C A A G C T A A T G T A T T T T A T T A T T G A G T G G A A A A T C T T A A T G A A
wsxfull.13.2.variant	2414	G T G A T T A C A A G C T A A T G T A T T T T A T T A T T G A G T G G A A A A T C T T A A T G A A
wsxfull.6.4.variant	2551	G A T G G T G A A A T A A A T G G C T T A G A A T C T C T T C A T C T G T T A A G A A G T A T T A
wsxfull.12.1.variant	2464	G A T G G T G A A A T A A A A T G G C T T A G A A T C T C T T C A T C T G T T A A G A A G T A T T A
wsxfull.13.2.variant	2464	G A T G G T G A A A T A A A A T G G C T T A G A A T C T C T T C A T C T G T T A A G A A G T A T T A
wsxfull.6.4.variant	2601	T A T C C A T G A T C A T T T T A T C C C C A T T G A G A A G T A C C A G T T C A G T C T T T A C C
wsxfull.12.1.variant	2514	T A T C C A T G A T C A T T T T A T C C C C A T T G A G A A G T A C C A G T T C A G T C T T T A C C
wsxfull.13.2.variant	2514	T A T C C A T G A T C A T T T T A T C C C C A T T G A G A A G T A C C A G T T C A G T C T T T A C C
wsxfull.6.4.variant	2651	C A A T A T T T A T G G A A G G A G T G G G A A A C C A A A G A T A A T T A A T A G T T T C A C T
wsxfull.12.1.variant	2564	C A A T A T T T A T G G A A G G A G T G G G A A A C C A A A G A T A A T T A A T A G T T T C A C T
wsxfull.13.2.variant	2564	C A A T A T T T A T G G A A G G A G T G G G A A A C C A A A G A T A A T T A A T A G T T T C A C T
wsxfull.6.4.variant	2701	C A A G A T G A T A T T G A A A A A C A C C A G A G T G A T G C A G G T T T A T A T G T A A T T G T
wsxfull.12.1.variant	2614	C A A G A T G A T A T T G A A A A A C A C C A G A G T G A T G C A G G T T T A T A T G T A A T T G T
wsxfull.13.2.variant	2614	C A A G A T G A T A T T G A A A A A C A C C A G A G T G A T G C A G G T T T A T A T G T A A T T G T

FIG. 3I

wsxfull.6.4.variant	2751	G C C A G T A A T T A T T T C C T C T T C C A T C T T A T T G C T T G G A A C A T T A T T A A T A T
wsxfull.12.1.variant	2664	G C C A G T A A T T A T T T C C T C T T C C A T C T T A T T G C T T G G A A C A T T A T T A A T A T
wsxfull.13.2.variant	2664	G C C A G T A A T T A T T T C C T C T T C C A T C T T A T T G C T T G G A A C A T T A T T A A T A T
wsxfull.6.4.variant	2801	C A C A C C A A A G A A T G A A A A A G C T A T T T T G G A A A G A T G T T C C G A A C C C C A A G
wsxfull.12.1.variant	2714	C A C A C C A A A G A A T G A A A A A G C T A T T T T G G A A A G A T G T T C C G A A C C C C A A G
wsxfull.13.2.variant	2714	C A C A C C A A A G A A T G A A A A A G C T A T T T T G G A A A G A T G T T C C G A A C C C C A A G
wsxfull.6.4.variant	2851	A A T T G T T C C T G G G C A C A A G G A C T T A A T T T T C A G A A G A G A C G G A C A T T C T
wsxfull.12.1.variant	2764	A A T T G T T C C T G G G C A C A A G G A C T T A A T T T T C A G A A G A T G T T C C G A A C C C C
wsxfull.13.2.variant	2764	A A T T G T T C C T G G G C A C A A G G A C T T A A T T T T C A G A A G C C A G A A A C G T T T G A
wsxfull.6.4.variant	2901	T T G A A G T C T A A T C A T G A T C A C T A C A G A T G A A C C C A A T G T G C C A A C T T C C C
wsxfull.12.1.variant	2814	A A G A A T T G T T C C T G G G C A C A A G G A C T T A A T T T T C A G A A G A T G C T T G A A G G
wsxfull.13.2.variant	2814	G C A T C T T T T A T C A A G C A T A C A G C A T C A G T G A C A T G T G G T C C T C T T T
wsxfull.6.4.variant	2951	A A C A G T C T A T A G A G T A T T A G A A G A T T T T T G A A G A A G G G C C G G A
wsxfull.12.1.variant	2864	C A G C A T G T T C G T T A A G A G T C A T C A C C A C T C C T A A T C T C A A G T A C C C A G G
wsxfull.13.2.variant	2864	T G G A G C C T G A A A C A A T T T C A G A A G A T A T C A G T G T T G A T A C A T C A T G G A A A
wsxfull.6.4.variant	3001	A T T C
wsxfull.12.1.variant	2914	G A C A C A A C A C T G C G G A A G G C C A C A G G T C C T C T G C A T A G G A A A C C A G A
wsxfull.13.2.variant	2914	A A T A A G A T G A G A T G A T G C C A A C A A C T G T G G T C T C T A C T T T C A A C A A C

FIG. 3J

[illegible]

FIG. 3K

wsxfull.13.2.variant 3514 TTAGGAACCTTCTAGTAAGAAGACTTTTGCATCTTACATGCCTCAATTCCA
wsxfull.13.2.variant 3564 AACTTGTCTACTCAGACTCATAGAATCATGGAAACAAGATGTGTGACC
wsxfull.13.2.variant 3614 TAACTGTGTAAATTTCACTGAAGAAACCTTCAGATTTGTGTTATAATGGGT
wsxfull.13.2.variant 3664 AATATAAAGTGTAATAGATTATAGTTGTGGTGGAGAGAGAAAGAAAC
wsxfull.13.2.variant 3714 CAGAGTCAAAATTTGAATAATTTGTTCCAAATGAATGTGTCTGTGTT
wsxfull.13.2.variant 3764 CTCTCTTAGTAACATAGACAAAATTTGAGAAAGCCTTCATAAGCCTAC
wsxfull.13.2.variant 3814 CAATGTAGACACGCTCTCTATTTTATTTCCCAAGCTCTAGTGGGAAGGTC
wsxfull.13.2.variant 3864 CCTTGTTTCCAGCTAGAAATAAGCCCAACAGACACCATCTTTTGTGAGAT
wsxfull.13.2.variant 3914 GTAAATGTTTTTTCAGAGGGCGTGTGTGTTTTACCTCAAGTTTTGTTTG
wsxfull.13.2.variant 3964 TACCAACACACACACACACATTCCTTAACACATGTCTGTGTGTTT
wsxfull.13.2.variant 4014 TGAGAGTATATTATGTATTTATATTTTGTGCTATCAGACTGTAGGATTTG
wsxfull.13.2.variant 4064 AGTAGGACTTTCCTAAATGTTTAAAGATAAACAGAAATTC

FIG. 3L

wsxfull.13.2.variant	1	M	I	C	Q	K	F	C	V	V	L	L	H	W	E	F	I	Y	V	I	T	A	F	N	L	S	Y	P	I	T	P	W	R	F	K	L	S	C	M	P	P	N	S	T	Y	D	Y	F	L	P	
mu.wsx.ecd	1	M	M	C	Q	K	F	Y	V	V	L	L	H	W	E	F	L	Y	V	I	A	A	L	N	L	A	Y	P	I	S	P	W	K	F	K	L	F	C	G	P	P	N	T	T	D	S	F	L	S	P	
wsxfull.13.2.variant	51	A	G	L	S	K	N	T	S	N	S	N	G	H	Y	E	T	A	V	E	P	K	F	N	S	S	G	T	H	F	S	N	L	S	K	T	T	F	H	C	C	F	R	S	E	O	D	R	N	C	S
mu.wsx.ecd	51	A	G	A	P	N	M	A	S	A	L	K	G	A	S	E	A	I	V	E	A	K	F	N	S	S	G	I	Y	V	P	E	L	S	K	T	V	F	H	C	C	F	G	M	E	O	G	O	N	C	S
wsxfull.13.2.variant	101	L	C	A	D	N	I	E	G	K	T	F	V	S	T	V	N	S	L	V	F	Q	I	D	A	N	W	N	I	Q	C	W	L	K	G	D	L	K	L	F	I	C	Y	V	E	S	L	F	K	N	
mu.wsx.ecd	101	A	L	T	D	N	T	E	G	K	T	L	A	S	V	V	K	A	S	V	F	R	Q	L	G	V	N	W	D	I	E	C	W	M	K	G	D	L	T	L	F	I	C	H	M	E	P	L	P	K	N
wsxfull.13.2.variant	151	L	F	R	N	Y	N	Y	K	V	H	L	L	Y	V	L	P	E	V	L	E	D	S	P	L	V	P	O	K	G	S	F	Q	M	V	H	C	N	C	S	V	H	E	C	C	E	C	L	V	P	V
mu.wsx.ecd	151	P	F	K	N	Y	D	S	K	V	H	L	L	Y	D	L	P	E	V	I	D	D	S	P	L	P	P	L	K	D	S	F	Q	T	V	Q	C	N	C	S	L	R	G	-	C	E	C	H	V	P	V
wsxfull.13.2.variant	201	P	T	A	K	L	N	D	T	L	L	M	C	L	K	I	T	S	G	V	I	F	Q	S	P	L	M	S	V	O	P	I	N	M	V	K	P	D	P	P	L	G	L	H	M	E	I	T	D	D	
mu.wsx.ecd	200	P	R	A	K	L	N	Y	A	L	L	M	Y	L	E	I	T	S	A	G	V	S	F	Q	S	P	L	M	S	L	O	P	M	L	V	V	K	P	D	P	P	L	G	L	H	M	E	V	T	D	D
wsxfull.13.2.variant	251	G	N	L	K	I	S	W	S	P	P	L	V	P	F	P	L	Q	Y	Q	V	K	Y	S	E	N	S	T	T	V	I	R	E	A	D	K	I	V	S	A	T	S	L	L	V	D	S	I	L	P	
mu.wsx.ecd	250	G	N	L	K	I	S	W	D	S	Q	T	M	A	P	F	P	L	Q	Y	Q	V	K	Y	L	E	N	S	-	T	I	V	R	E	A	E	I	V	S	A	T	S	L	L	V	D	S	V	L	P	

FIG. 4A

wsxfull.13.2.variant	301	GSSYEVOVR	GKRLDGP	GIIWSDWS	TPR	VFTTQDV	IYFPPKILTSVGSN	VSF						
mu.wsx.ecd	299	GSSYEVOVR	SKRLDGS	GVWSDWS	SPQ	VFTTQDV	VYFPPKILTSVGSNASF							
wsxfull.13.2.variant	351	HC IYK	ENK	IVP	SK E	IVW	MNLA EKIP	OSQYDV	VSDH	VSKVTF	FNL	NETK		
mu.wsx.ecd	349	HC IYK	ENQ	IVS	SKQ	IVW	RNLA EKIP	EIQYSI	VSDR	VSKVTF	SNL	KATR		
wsxfull.13.2.variant	401	PRGKFTYDAVYCCNE	HE	CHHRYAEL	YVIDVN	INIS	CETDGYLT	KMTCRWS						
mu.wsx.ecd	399	PRGKFTYDAVYCCNE	QA	CHHRYAEL	YVIDVN	INIS	CETDGYLT	KMTCRWS						
wsxfull.13.2.variant	451	TSTIOSL	AEST	OLRYHRS	SLYC	SDI	PSIHP	ISEPK	DCYLQSDG	FYECIF				
mu.wsx.ecd	449	PSTIOSL	VGST	OLRYHR	CSLYC	PDS	PSIHP	TSEPK	TASYRET	AFMNVFS				
wsxfull.13.2.variant	501	QPI	FL	LSGYTMW	I	RINHSL	GSLS	DS	PPTCVLP	DSVVKPLPPS	S	VKAEIT	IN	
mu.wsx.ecd	499	SOS	F	YVLA	IQCGF	R	RINHSL	GSLS	DS	PPTCVLP	DSVVKPLPPS	N	VKAEIT	VN
wsxfull.13.2.variant	551	GLLK	I	SWEKPV	FPENN	LOFO	IRYGL	SGKE	VQWK	MY	EVY	DAKSKS	VSL	PV
mu.wsx.ecd	549	TGLLK	V	SWEKPV	FPENN	LOFO	IRYGL	SGKE	IOWKT	THE	VF	DAKSKS	ASL	LV

FIG. 4B

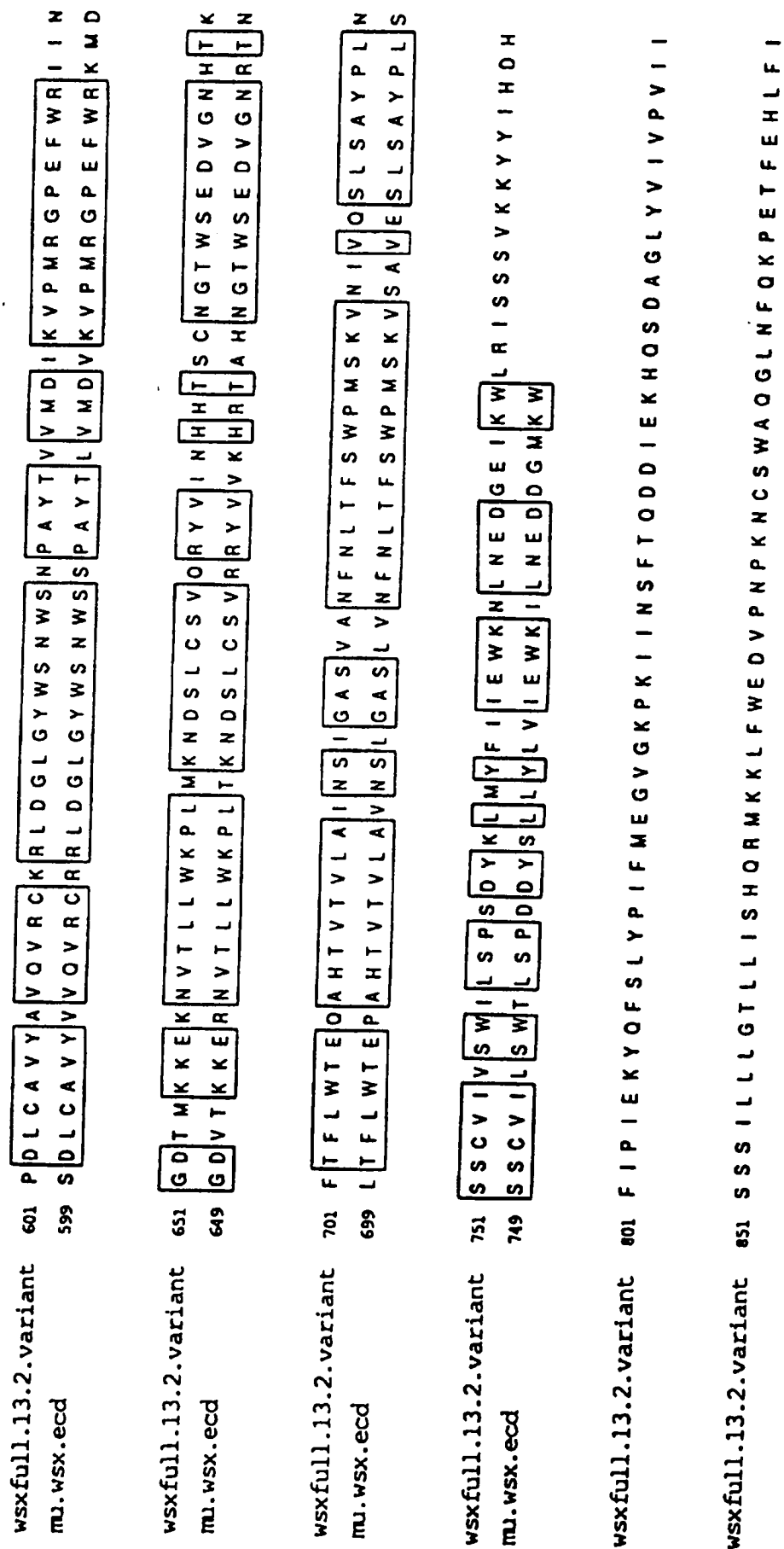


FIG. 4C

30/85

wsxfull.13.2.variant 901 KHTASVTCGPLLEPETISEDISVDTSWKNKDEMMPTTVVSLSTTDLEK

wsxfull.13.2.variant 951 GSVCSIDQFNSVNFSEAEGETEVTYEDESQRQPFVKYATLISNSKPSETGE

wsxfull.13.2.variant 1001 EQGLINSSVTKCFSSKNSPLKDSFSNSSWEIEAQAFFILSDQHPNII SPH

wsxfull.13.2.variant 1051 LTFSEGLDELLKLEGNFPEENNDKKSIIYLVGVTSIKKRESGVLLTDKSRV

wsxfull.13.2.variant 1101 SCPFPAPCLFTDIRVLQDSCSHFVENNINLGTSSKKTFASYMPOFOTCST

wsxfull.13.2.variant 1151 QTHKIMENKMCOLT V

FIG. 4D

nu.wsx.ecd 1 GGGCCCCCCTCGAAGTCGACGGTATCGATAAGCTTGATATCGAATTCCG

 nu.wsx.ecd 51 GCCGGGACACAGGTGGGACACTCTTTAGTCCTCAATCCCTGGCGCGAGG

 nu.wsx.ecd 101 CCACCCAAAGGCAACGGCAGGACGGCGTTTGGGGACCAAGCAGCAGAC

 nu.wsx.ecd 151 TGGGGCGGTACCTGCGGAGAGCCACGCAACTTCTCCAGGCCCTCTGACTAC

 nu.wsx.ecd 201 TTTGGA AACTGCCCGGGGCTGCGACATCAACCCCTTAAGTCCCGGAGGCG

 nu.wsx.ecd 251 GAAAGAGGGTGGGTGGTTTGAAAGACACAAAGGAAGAAAATGTGCTGTG

 nu.wsx.ecd 301 GGGCGGGTTAAGTTTCCCACCCCTCTTCCCCTTCCCGAGCAATTAGAAA

 nu.wsx.ecd 351 CAAACA AATAGAAAAGCCAGCCCTCCGGCCAAACC
 #sxfull.13.2.variant 1 GAATTCTCAGTCCGAC

FIG. 5A

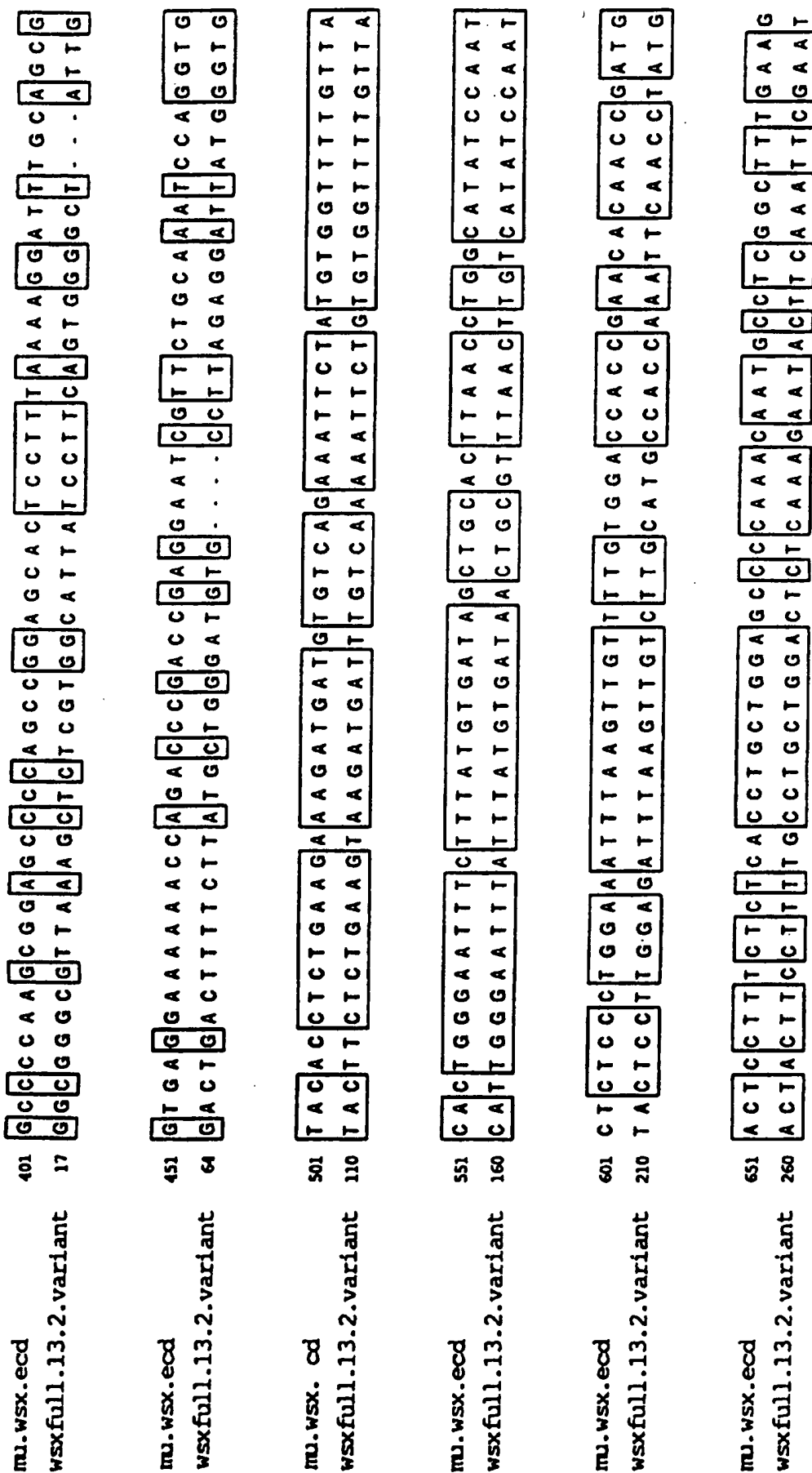


FIG. 5B

ml.wsx.ecd	1001	T T A T A T G A	T C T G C C T G A A G T	C A T A G A T	G A T T C	C C T C T G	C C C C A	C T G A A
wsx.full.13.2.variant	610	T T A T A T G T	T C T G C C T G A A G T	G T T A G A A	G A T T C	A C C T C T G	G T T C C C A	A A A
ml.wsx.ecd	1051	A G A C A G C T	T T T C A G A C T G T	C C A A T G C A A C	T G C A G T C	T T C G G G	- -	G A T G T G
wsx.full.13.2.variant	660	A G C A G T	T T T C A G A T G G T	T C A C T G C A A T	T G C A G T G	T T C A T G	A A T G	T T G T G
ml.wsx.ecd	1098	A A T G T C A	T G T G C C A G T A C C C A	G A G C C A A A C T C A A C	T A C G	C T C T	T C T G A T G	
wsx.full.13.2.variant	710	A A T G T C T	T G T G C C T G T G C C A A	C A G C C A A A C T C A A C	G A C A	C T C T C	C T T A T G	
ml.wsx.ecd	1148	T A T T T G G A	A A T C A C A T C T G C C G G T	G T G A G	T T T T	C A G T C A C C T C T	G A T G T C	
wsx.full.13.2.variant	760	T G T T T G A A	A A T C A C A T C T G G T G G A	G T A A T T T T	C A G T C A C C T C T	A A T G T C		
ml.wsx.ecd	1198	A C T G C A G C C C C A T	G C T T G T T G T G A A A C C C	G A T C C A C C C	T T A G G T T T G C A T A			
wsx.full.13.2.variant	810	A G T T C A G C C C C A T	A A A T A T G G T G A A G C C T	G A T C C A C C A	T T A G G T T T G C A T A			
ml.wsx.ecd	1248	T G G A A G T C A C A G A T G A T G G T A A T T T A A G A T T T C T T G G A	C A G C C A A A C A					
wsx.full.13.2.variant	860	T G G A A A T C A C A G A T G A T G G T A A T T T A A G A T T T C T T G G T C	C A G C C A C A C A					

FIG. 5D

mu.wsx.ecd	1298	A T G G C	A C C A T T T C C G	C T T C A A T A T C A	G G T G A A A T A T T	T A G A G A A T T C T A C
	wsx.full.13.2.variant	910	T T G G T	A C C A T T T C C A	C T T C A A T A T C A	A G T G A A A T T C T A C
mu.wsx.ecd	1348	A A . . .	T T G T	A A G A G A G	G C T G	C T G
	wsx.full.13.2.variant	960	A A C A G T T A T	C A G A G A A	G C T G A C A	A G A T T G T C T C A G C T A C A T C T C T G C T A G
mu.wsx.ecd	1395	T A G A C A G T G T	G C T T C C T G G A	T C T T C A	T A T G A G G T C	C A G G T G A G G A G C A A G
	wsx.full.13.2.variant	1010	T A G A C A G T A T A	C T T C C T G G G	T C T T C G	T A T G A G G T T C A G G T G A G G G C A A G
mu.wsx.ecd	1445	A G A C T G G A T G G T T	C A G G A G T	C T G G A G T G A C T G G A G T T	C A C C T C A A	A G T C T T
	wsx.full.13.2.variant	1060	A G A C T G G A T G G C C	C A G G A A T	C T G G A G T G A C T G G A G T A C T	C C T C G T G T C T T
mu.wsx.ecd	1495	T A C C A C A C A A G A T G T T G	T G T A T	T T T C C A C C C	C A A A A T T C T G A C T	A G T G T T G
	wsx.full.13.2.variant	1110	T A C C A C A C A A G A T G T C A T A T A C T	T T C C A C C T	T A A A A T T C T G A C A	A G T G T T G
mu.wsx.ecd	1545	G A T C G A A T G C T T C C	T T T C A T	T G C A T C T A C A A A	A A C G A A A A C C	A G A T T G T C
	wsx.full.13.2.variant	1160	G G T C T A A T G T T T C T	T T T C A C T G C A T C T A T A A G A	A G A A A A C A	A G A T T G T T

FIG. 5E

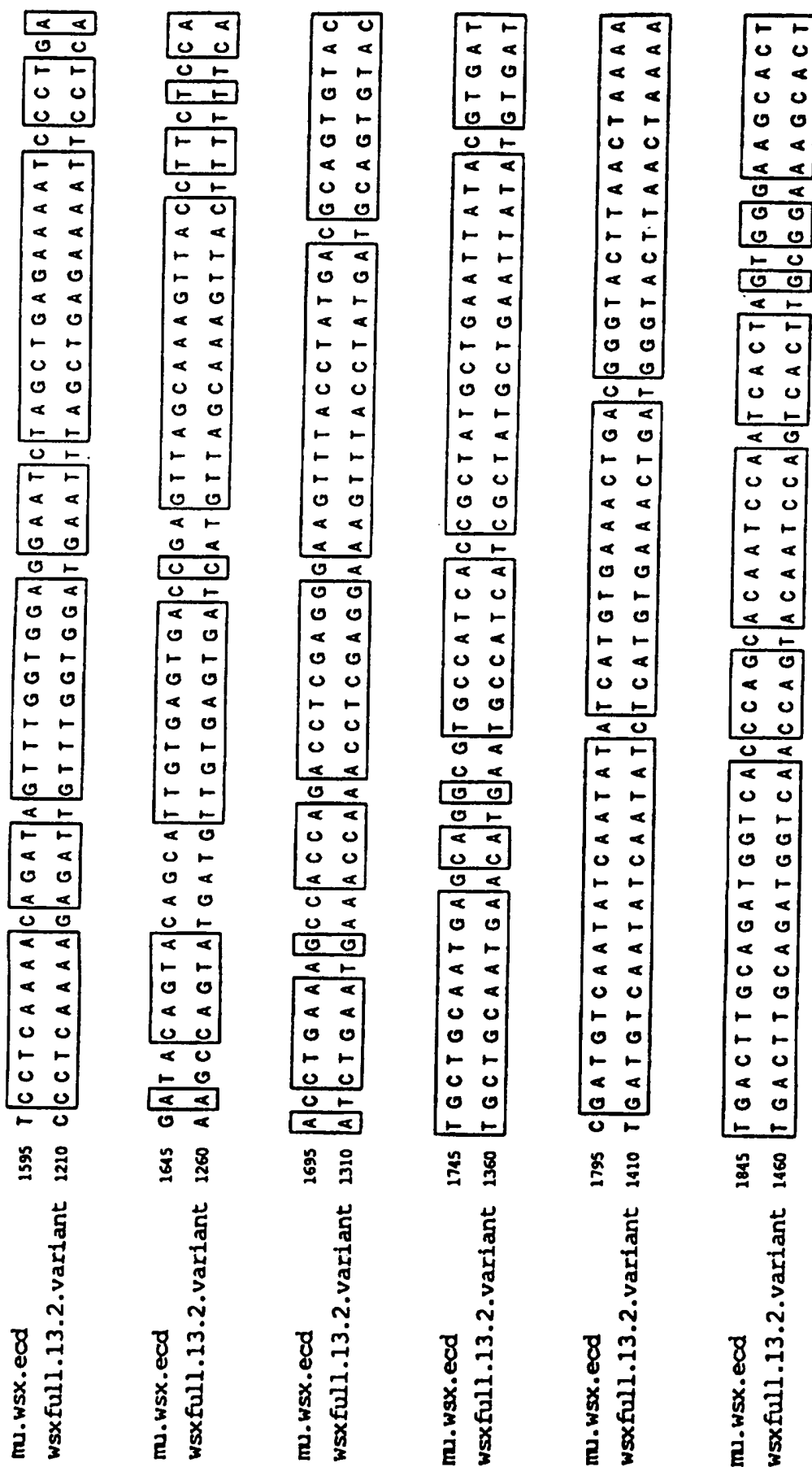


FIG. 5F

ml.wsx.ecd
wsx.full.13.2.variant

mj.wsx.ecd
 1945
 T A T T C A T C C T A C G T C T G A G C C C A A A - A C T G C G T C T T A C A G A G A G A C G G C T T
 wsx.full.13.2.variant 1560
 T A T T C A T C C C A T A T C T G A G C C C A A A G A T T G C T A T T T G C A G A G T G A T G G T T

Accession	Position	Sequence
ml.wsx.ecd	1994	TTTATGAATGTTTTCAGCCAATCTTCTATTATCTGGCTATACAATG
	1994	TTTATGAATGTTTTCAGCCAATCTTCTATTATCTGGCTATACAATG
wsx.full.13.2.variant	1610	TTTATGAATGTTTTCAGCCAATCTTCTATTATCTGGCTATACAATG
	1610	TTTATGAATGTTTTCAGCCAATCTTCTATTATCTGGCTATACAATG

mu.wsx.ecd
2044
wsx.full.13.2.variant
1660

Accession	Position	Sequence
mu.wsx.ecd	2094	GTGTCCTTCCCTGACTCCGTAAGTAAACCACTACCTCCATCTAACGTAA
	1709	GTGTCCTTCCCTGACTTCGTGTGTAAGCCACTGCCCTCCATCCAGTGTGAA

mu.wsx.ecd
2144
GCAGAGATTACTGTAAACACCTGGATTATTGAAAGTATCTTGGGAAAGCC

FIG. 5G

mu.wsx.ecd	2194	AGTCTTTCCG	GAGAAATAACCTTCAATTCCAGATTTCGA	TATGGC	TTAAGTG
wsxfull.13.2.variant	1809	AGTCTTTCCA	GAGAAATAACCTTCAATTCCAGATTTCGC	TATGGT	TTAAGTG
mu.wsx.ecd	2244	GAAAGAA	TACCAATGGAAGA	CACATGAGGT	GATGCAAA
wsxfull.13.2.variant	1859	GAAAGAA	TACCAATGGAAGA	TGTATGAGGT	GATGCAAA
mu.wsx.ecd	2294	TCTGCCAGC	CTGCTGTGT	CAGACCT	TGTGTCAGTCTATG
wsxfull.13.2.variant	1909	TCTGTCAGT	CTCCAGTTC	CAGACCT	TGTGTCAGTCTATG
mu.wsx.ecd	2344	TCGCTGCCG	CGGTCGGT	TGATGGACT	AGGATATTGGAGTAATTGGAGCA
wsxfull.13.2.variant	1959	CGCTGTAA	GAGGCTA	GATGGACT	AGGATATTGGAGTAATTGGAGCA
mu.wsx.ecd	2394	CAGCCTAT	ACGCCTTGTCATGGAT	GTA	TA
wsxfull.13.2.variant	2009	CAGCCTACA	CAGTGTGTCTATGGAT	TA	TA
mu.wsx.ecd	2444	TTTGGAGAA	AAATGGG	GATGGG	GATGGG
wsxfull.13.2.variant	2059	TTTGGAGAA	TAATTAATGGG	GATGGG	GATGGG

FIG. 5H

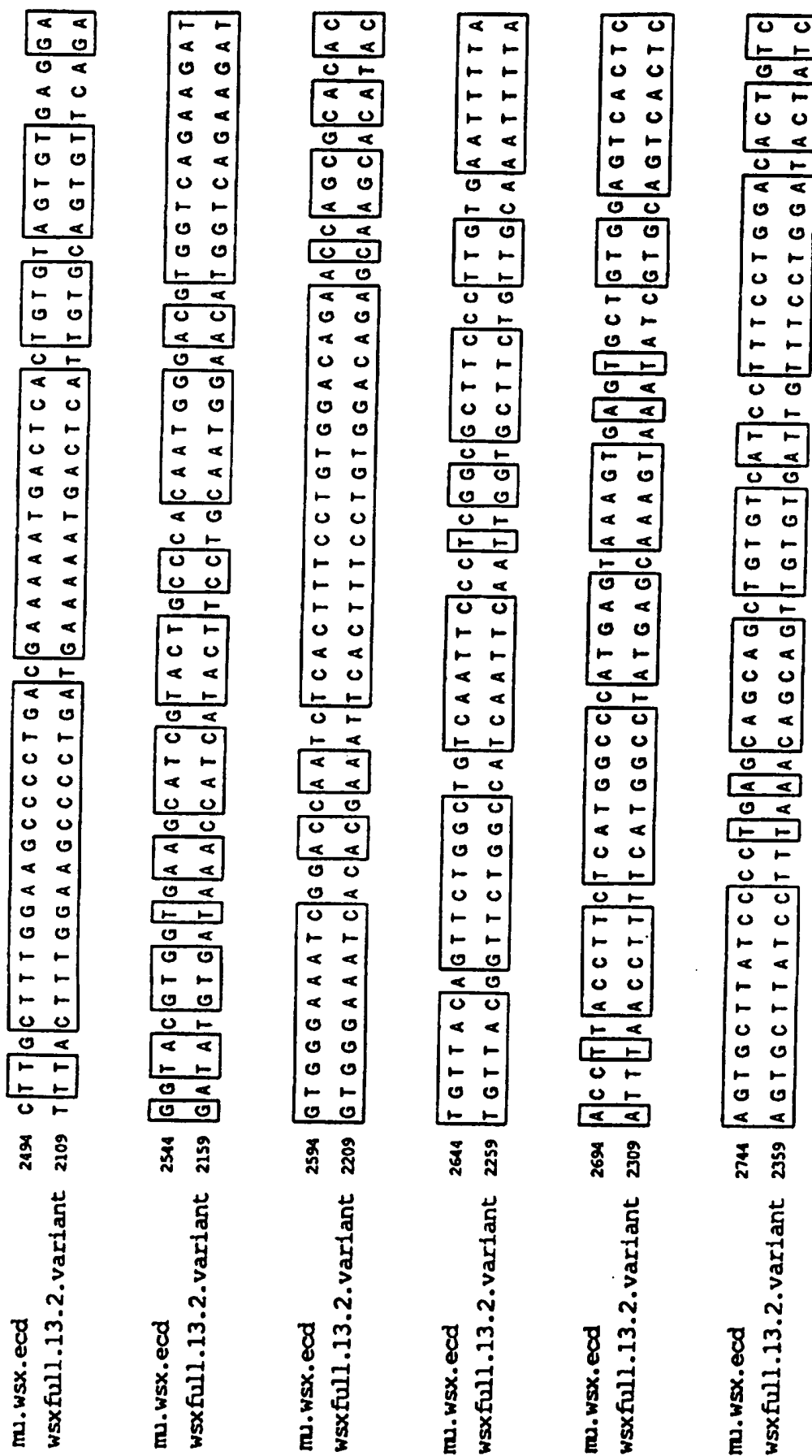


FIG. 5I

nu.wsx.ecd 2794 ACC TGA TGATTATAGTCTGTTAATCTGGAAGATCTTA
 wsxfull.13.2.variant 2409 ACC CAG TGATTACAGCTAAATGTAATTTATTTGGAAGATCTTA

nu.wsx.ecd 2844 ATGAAGATGATGGAAATGAAGTGGCT
 wsxfull.13.2.variant 2459 ATGAAGATGGTGAATAAATGGCTTAGAATCTCTCATCTGTTAAGAAG

wsxfull.13.2.variant 2509 TATTATATCCATGATCATTTTATCCCCATTGAGAAAGTACCAAGTTCAGTCT

wsxfull.13.2.variant 2559 TTACCCCAATATTTATGGAAAGGAGTGGGAAACCAAGATAATTATAAGTT

wsxfull.13.2.variant 2609 TCACCTCAAGATGATATTGAAAAACACCAAGAGTGATGCAGGTTTATATGTA

wsxfull.13.2.variant 2659 ATTGTGCCAGTAATTATTTCTCTCTCCATCTTATTTGCTTGGAAACATTATT

wsxfull.13.2.variant 2709 AATATCACACCAAGAAATGAAAAAGCTATTTTGGGAAGATGTTCCGAACC

wsxfull.13.2.variant 2759 CCAAGATTGTTCCTGGGCACAAGGACTTAATTTTCAGAAAGCCAGAAACG

FIG. 5J

wsxfull.13.2.variant 2809 TTTGAGCATCTTTTATCAAGCATACAGCATCAGTGACATGTGGTCCCTCT
wsxfull.13.2.variant 2859 TCTTTTGGAGCCTGAACAATTTCAGAAAGATATCAGTGTGATACATCAT
wsxfull.13.2.variant 2909 GGAAAAATAAGATGAGATGATGCCAACAACTGTGGTCTCTACTTTCA
wsxfull.13.2.variant 2959 AACACAGATCTTGAAAAGGGTTCTGTTTGTATTAGTGACCAGTTCAACAG
wsxfull.13.2.variant 3009 TGTTAACTTCTCTGAGGCTGAGGGTACTGAGGTAACTATGAGGACGAAA
wsxfull.13.2.variant 3059 GCCAGAGACAACCTTTTGTTAAATACGCCACGGCTGATCAGCAACTCTAAA
wsxfull.13.2.variant 3109 CCAAGTGAAACTGGTGAAGAACAAAGGGCTTATAAATAGTTCAGTCACCAA
wsxfull.13.2.variant 3159 GTGCTTCTCTAGCAAAAATTCTCCGTTGAAGGATTCTTTCTCTAATAGCT
wsxfull.13.2.variant 3209 CATGGGAGATAGAGGCCCAAGGCATTTTATATTATCAGATCAGCATCCC

FIG. 5K

wsxfull.13.2.variant 3259 AACATAATTTCACCACACCTCACATTCTCAGAGGATTGGATGAACCTTTT
wsxfull.13.2.variant 3309 GAAATTGGAGGGGAAATTTCCCTGAAGAAATAATGATAAAAGTCTATCT
wsxfull.13.2.variant 3359 ATTATTTAGGGGTCACCTCAATCAAAAGAGAGAGAGTGGTGTGCTTTTG
wsxfull.13.2.variant 3409 ACTGACAAGTCAAGGGTATCGTGCCCATTCCCAGCCCCCTGTTTATTCAC
wsxfull.13.2.variant 3459 GGACATCAGAGTTCTCCAGGACAGTTGCTCACACTTTGTAGAAATAATA
wsxfull.13.2.variant 3509 TCAACTTAGGAACCTTCTAGTAAGAAGACTTTTGCACTCTACATGCCCTCAA
wsxfull.13.2.variant 3559 TTCCAACCTTGTTCTACTCAGACTCATAGATCATGGAAACAAGATGTG
wsxfull.13.2.variant 3609 TGACCTAACTGTGTAAATTCACCTGAAGAAACCTTCAGATTTGTGTTATAA
wsxfull.13.2.variant 3659 TGGGTAAATATAAGTGTAATAGATTATAGTTGTGGGTGGAGAGAGAAAA

FIG. 5L

wsxfull.13.2.variant 3709 G A A C C A G A G T C A A A T T T G A A A T A A T T G T T C C A A A T G A A T G T T G T C T G T

wsxfull.13.2.variant 3759 T T G T T C T C T C T T A G T A A C A T A G A C A A A A A T T T G A G A A A G C C T T C A T A A G

wsxfull.13.2.variant 3809 C C T A C C A A T G T A G A C A C G C T C T T C T A T T T T A T T C C C A A G C T C T A G T G G G A

wsxfull.13.2.variant 3859 A G G T C C C T T G T T T C C A G C T A G A A A T A A G C C C A A C A G A C A O C A T C T T T T G T

wsxfull.13.2.variant 3909 G A G A T G T A A T T G T T T T T C A G A G G G C G T G T T T T A C C T C A A G T T T T G

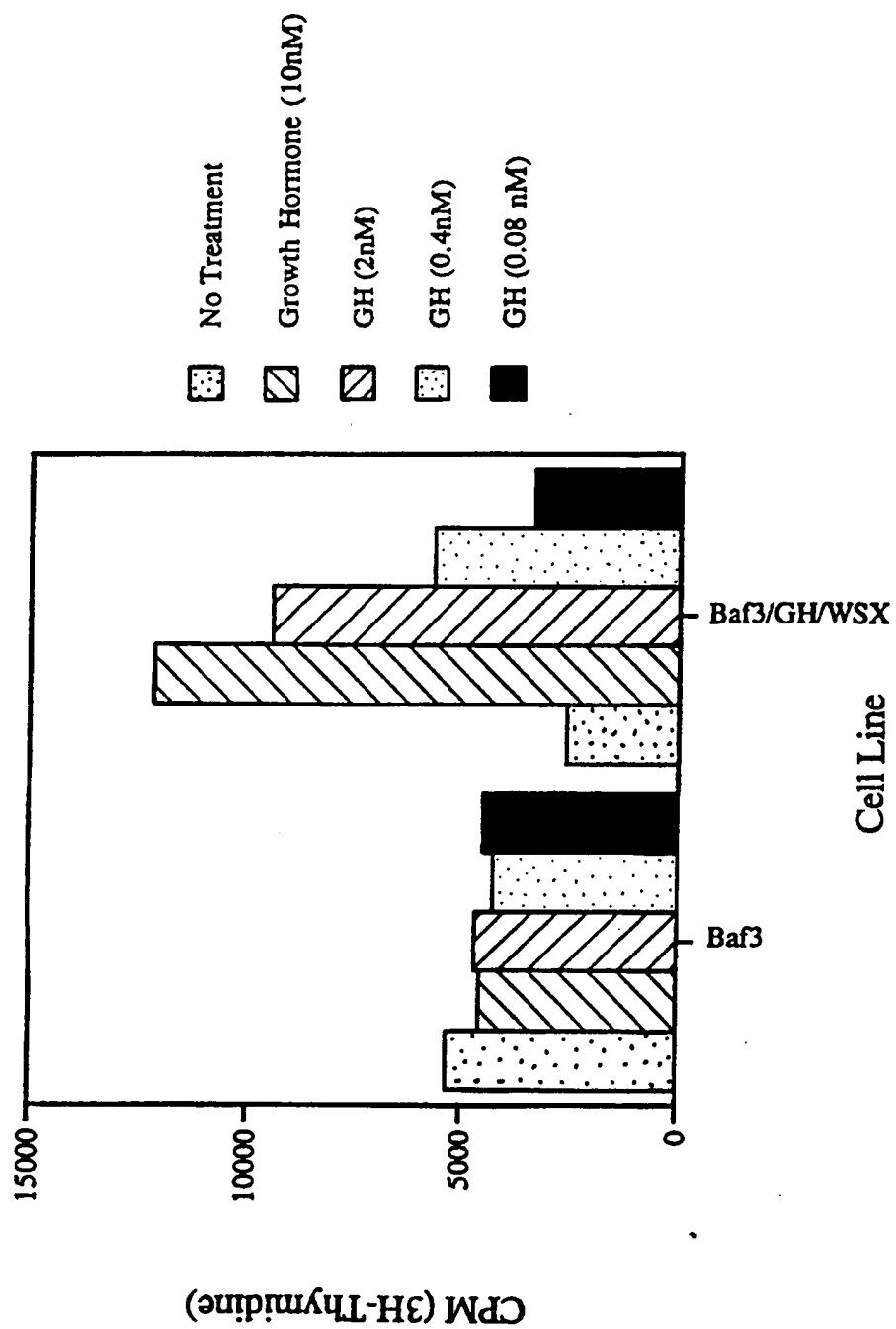
wsxfull.13.2.variant 3959 T T T T G T A C C A A C A C A C A C A C A C A T T C T T A A C A C A T G T C C T T G T G

wsxfull.13.2.variant 4009 T G T T T T G A G A G T A T A T T A T G T A T T T A T A T T T T G T G C T A T C A G A C T G T A G G

wsxfull.13.2.variant 4059 A T T T G A A G T A G G A C T T T C C T A A A T G T T T A A G A T A A C A G A A T T C

FIG. 5M

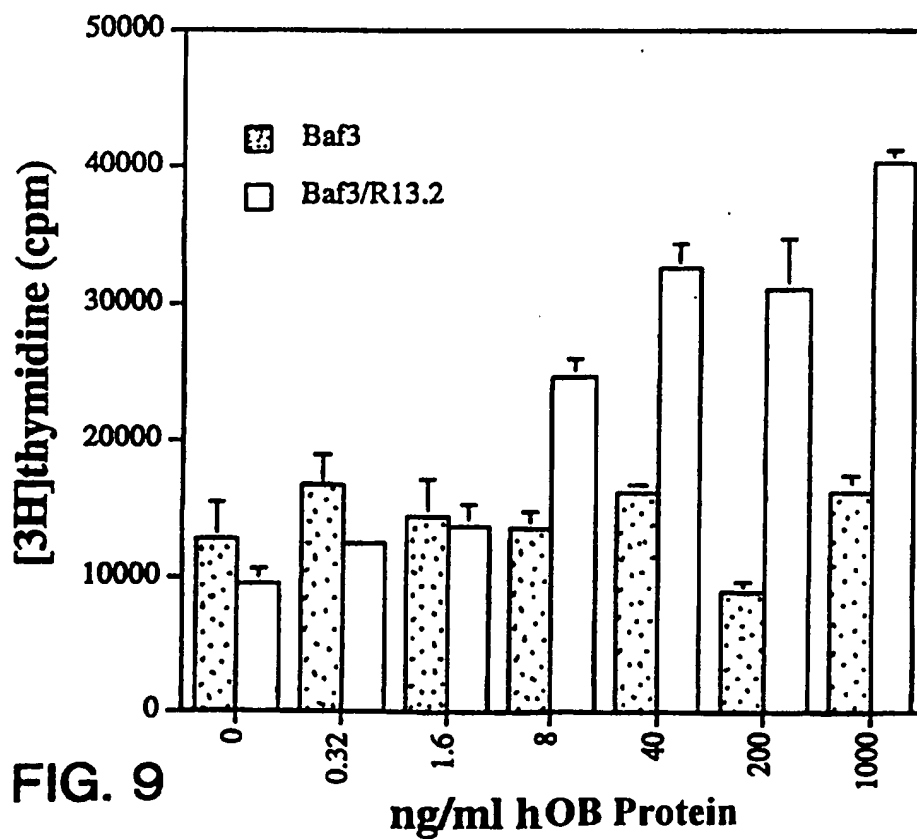
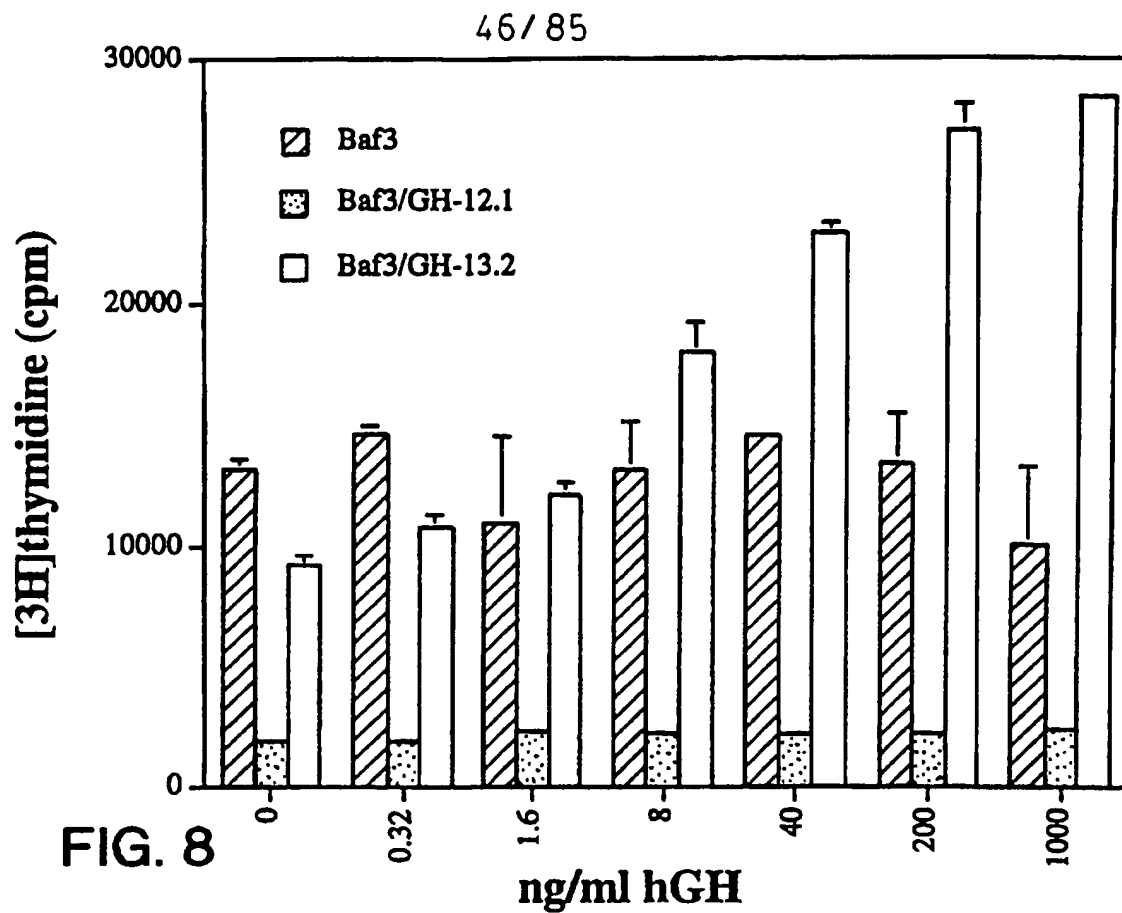
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Cell Line
FIG. 6

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Murine			
-213	Sense:	GGGTTAAGTTTCCCACCC	(SEQ ID NO:9)
	Antisense:	GGGTGGGAAACTTAACCC	(SEQ ID NO:10)
	Scrambled:	AGGATACAGTGGGATCCC	(SEQ ID NO:11)
-99	Sense:	GCCCGAGCACTCCTTTAA	(SEQ ID NO:12)
	Antisense:	TTAAAGGAGTGCTCCCGC	(SEQ ID NO:13)
	Scrambled:	GAGCGGCCCTGTTAGATA	(SEQ ID NO:14)
-20	Sense:	GTATACACCTCTGAAGAA	(SEQ ID NO:15)
	Antisense:	TTCTTCAGAGGTGTACAC	(SEQ ID NO:16)
	Scrambled:	ATGCGAGGCTACTTCTAT	(SEQ ID NO:17)
+84	Sense:	CTCTCCCTGGAAATTTAA	(SEQ ID NO:18)
	Antisense:	TTAAATTTCCAGGGAGAG	(SEQ ID NO:19)
	Scrambled:	ATTTGAAGGAGTTAAGCC	(SEQ ID NO:20)
+211	Sense:	AATTTAATTCAAGTGGTA	(SEQ ID NO:21)
	Antisense:	TACCAGTTGAATTAAATT	(SEQ ID NO:22)
	Scrambled:	GTATCACTTCATAATATA	(SEQ ID NO:23)
Human			
5L	Sense:	GATGGTCAGGGTGAAGT	(SEQ ID NO:24)
	Antisense:	CAGTTCACCCTGACCATC	(SEQ ID NO:25)
	Scrambled:	GAGGCGAATGTGCGGATT	(SEQ ID NO:26)
+85	Sense:	CTTAAATCTCCAAGGAGT	(SEQ ID NO:27)
	Antisense:	ACTCCTTGGAGATTTAAG	(SEQ ID NO:28)
	Scrambled:	AAGTCTTAAGCCAGACTT	(SEQ ID NO:29)
-47	Sense:	TCTAAGGCACATCCCAGC	(SEQ ID NO:30)
	Antisense:	GCTGGGATGTGCCTTAGA	(SEQ ID NO:31)
	Scrambled:	CGCAATGAATTGACCCCC	(SEQ ID NO:32)
-20	Sense:	TACTTCAGAGAAGTACAC	(SEQ ID NO:33)
	Antisense:	GTGTACTTCTCTGAAGTA	(SEQ ID NO:34)
	Scrambled:	GAATCACGGTAACTATCA	(SEQ ID NO:35)
+185	Sense:	CAGCTGTCTCATAATGTC	(SEQ ID NO:36)
	Antisense:	GACATTATGAGACAGCTG	(SEQ ID NO:37)
	Scrambled:	TTCGTCAAGCCATCTGAT	(SEQ ID NO:38)

FIG. 7



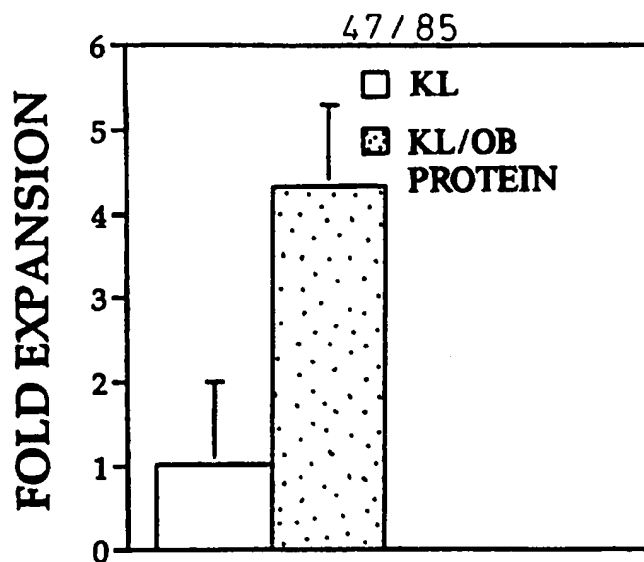


FIG. 10A

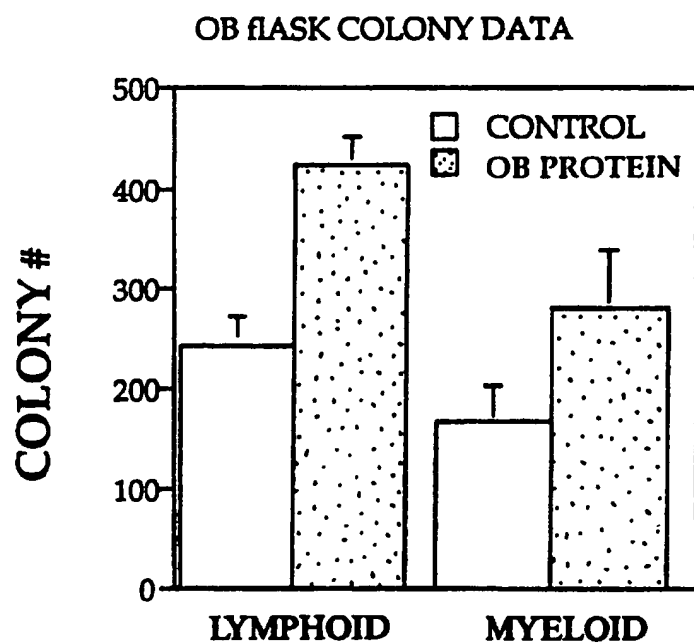


FIG. 10B

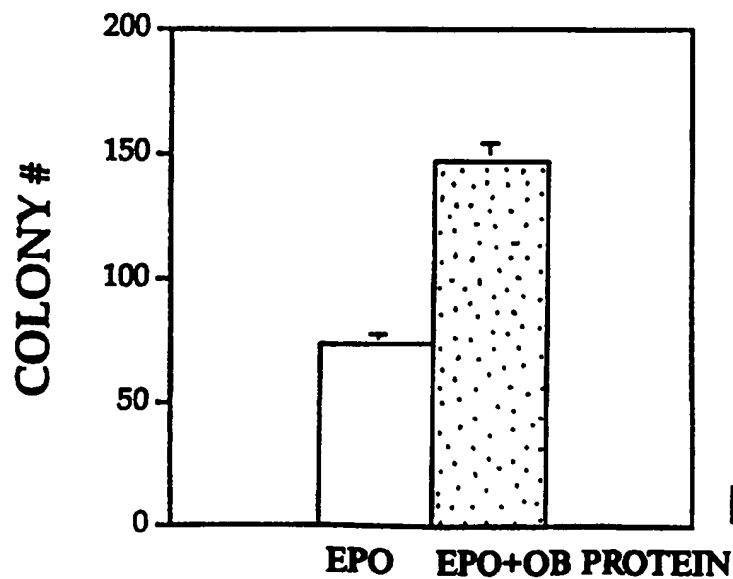


FIG. 10C

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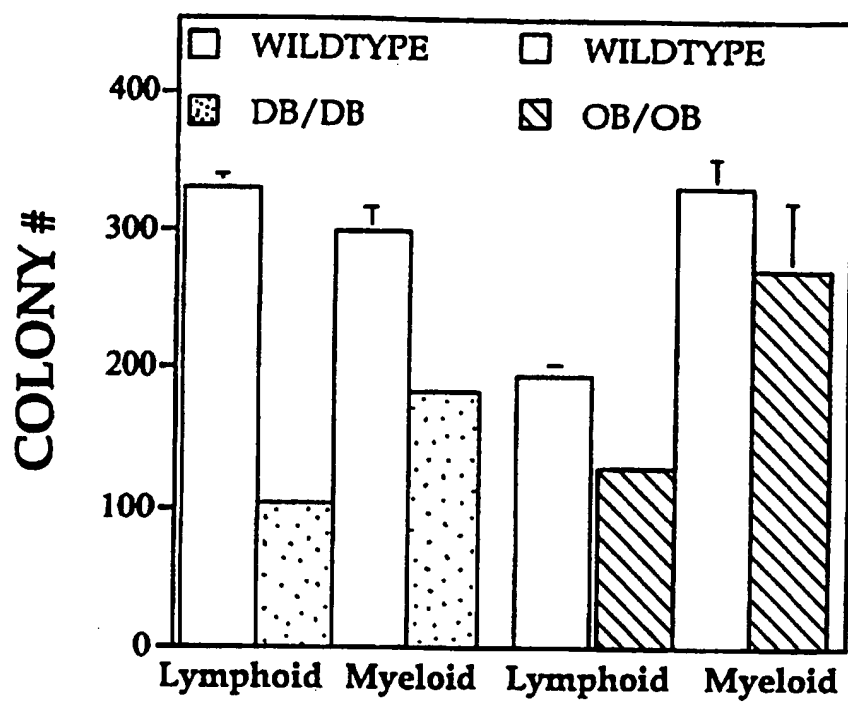


FIG. 11

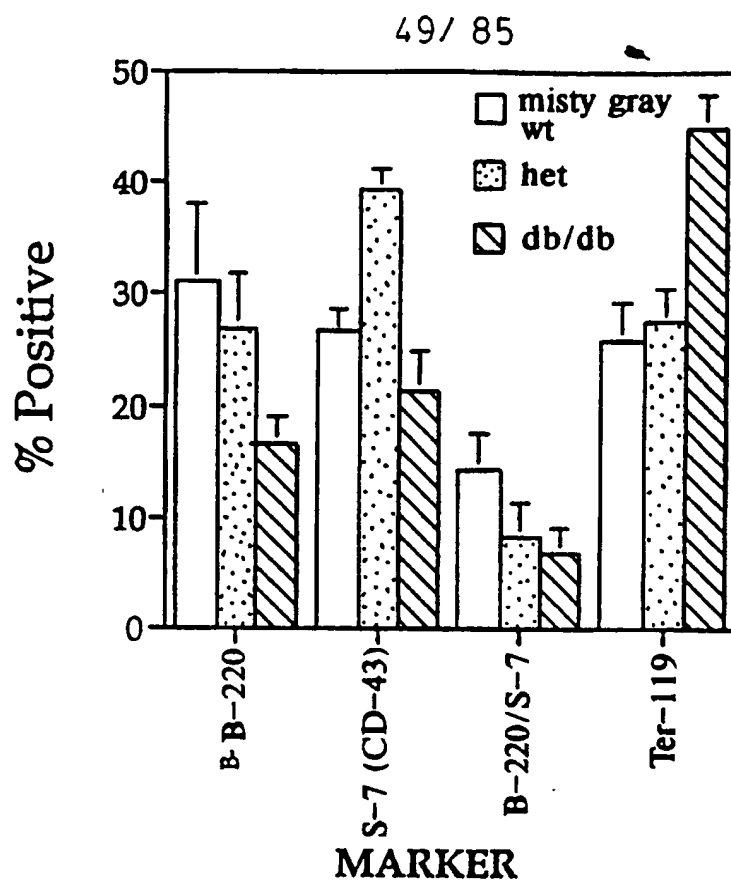


FIG. 12A

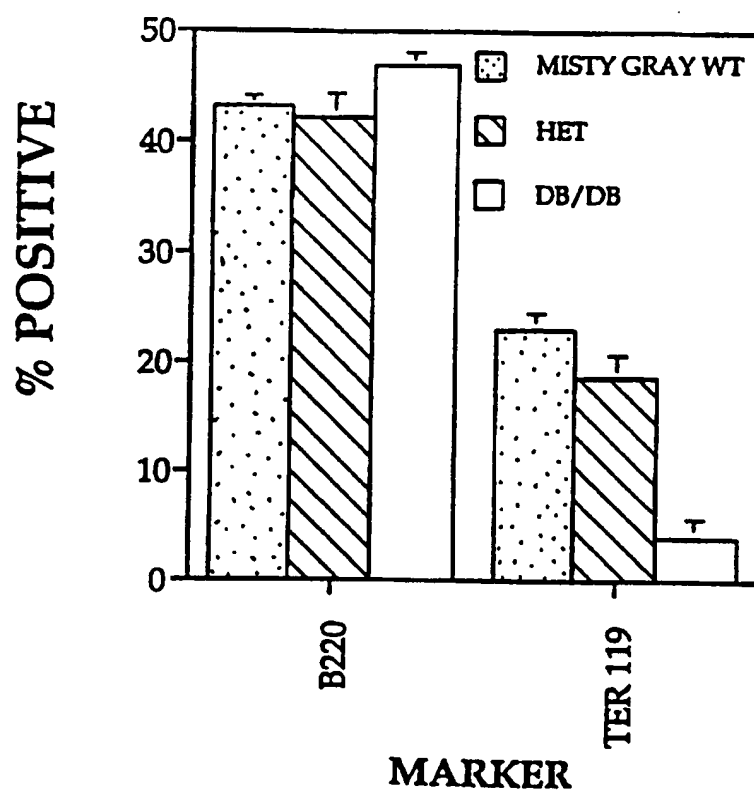


FIG. 12B

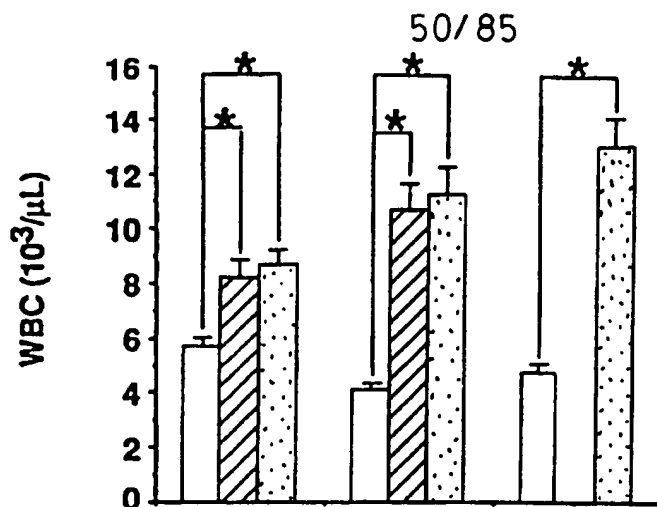


FIG. 13A

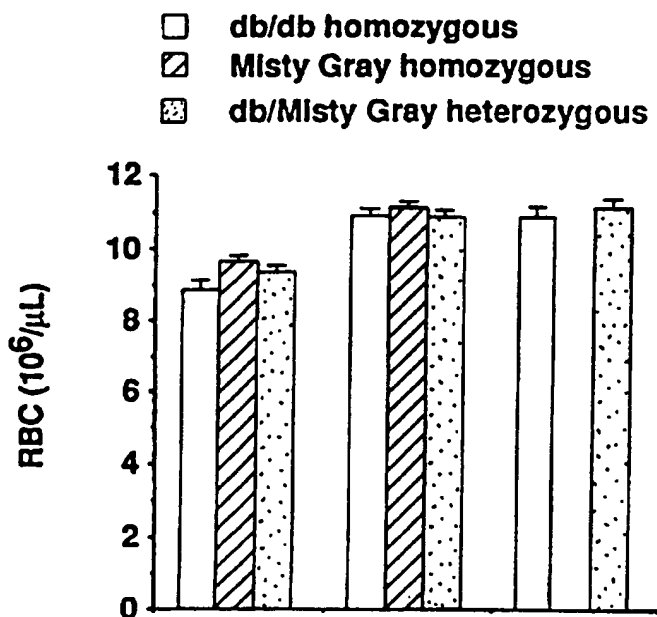


FIG. 13B

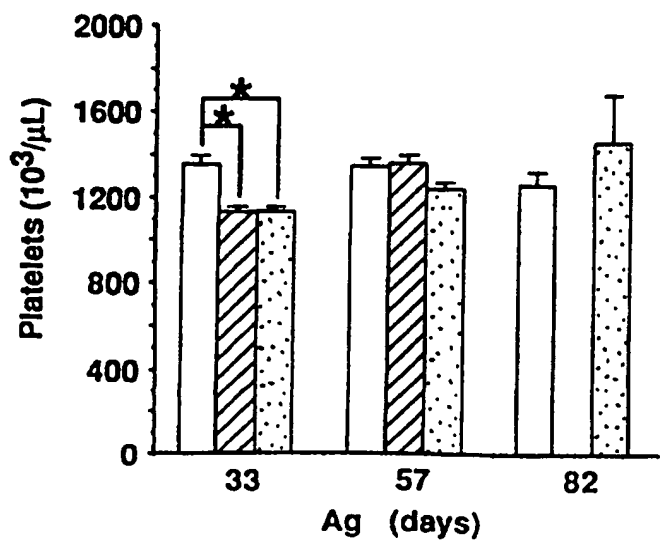
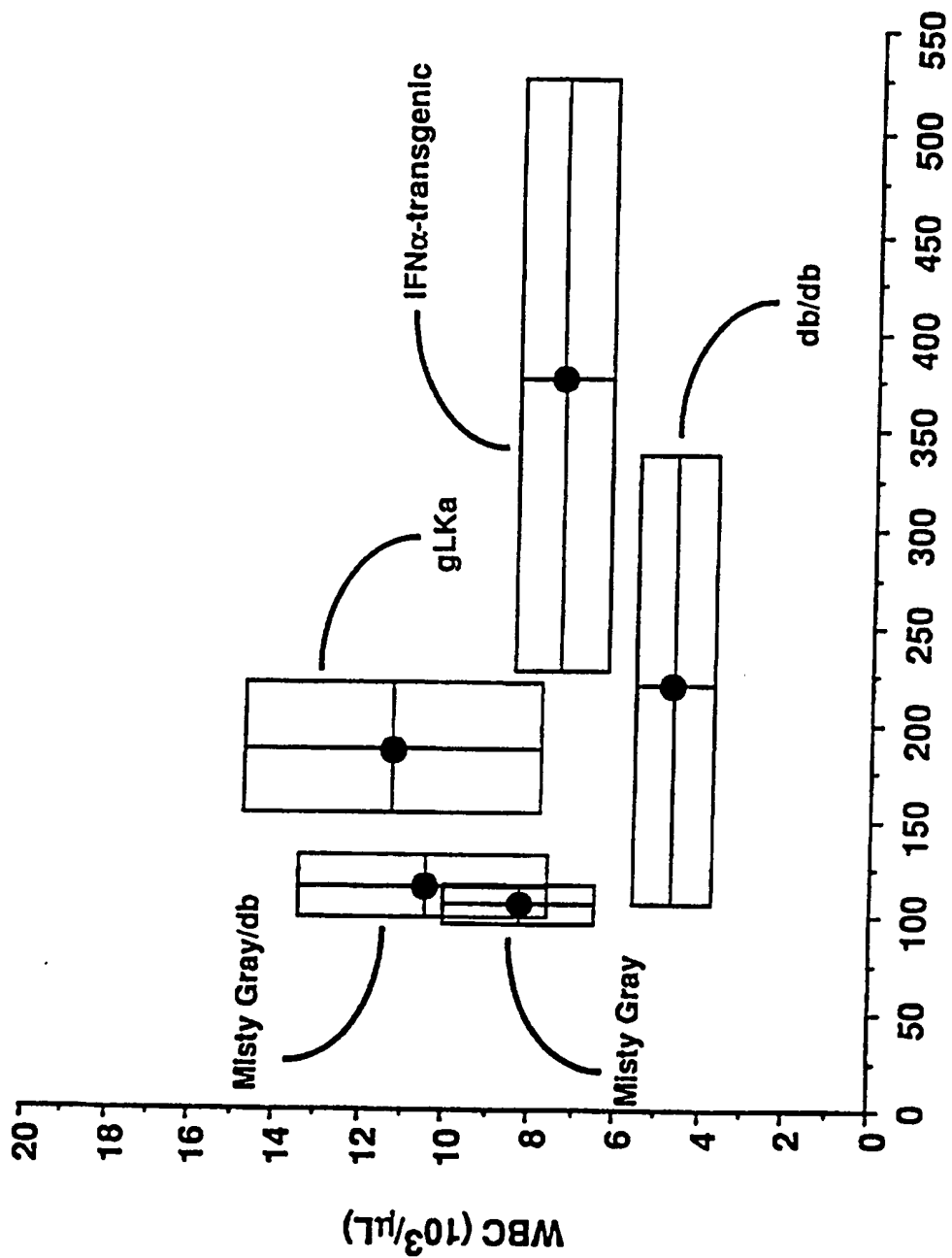


FIG. 13C



Blood Glucose (mg/dL)

FIG. 14

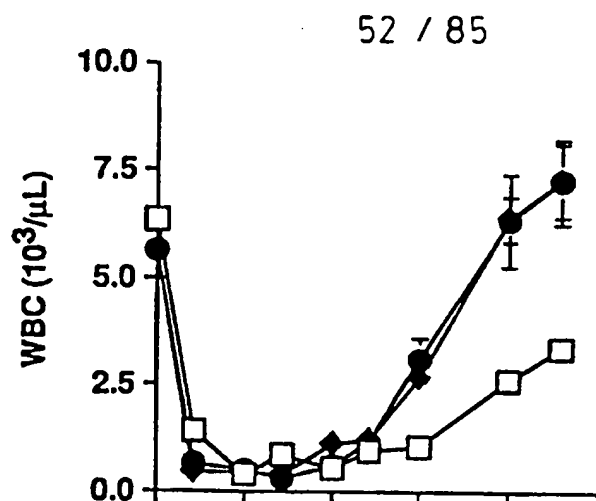


FIG. 15A

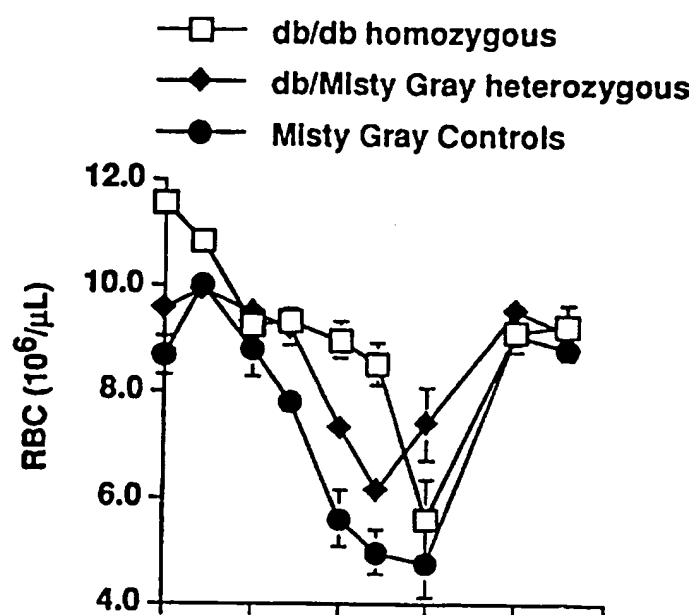


FIG. 15B

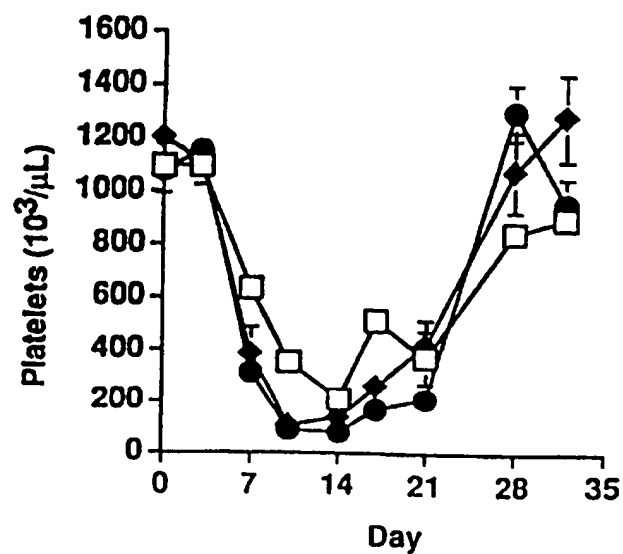


FIG. 15C

```

> sites: std
> length: 7127 (circular)

      aluI
      satI
      sacI
      hglIII
      hglAI/aspHI
      eciI36II
      bspI286
      bsiHKA1
      bayI
      banII
      taqI
      rmaI   tru9I
      maeI   mseI
      speI   aseI/asnI/vspI
      1 TTGAGCTCG CCGACATTC ATTATGACT AGTATTAT AGTAATCAAT TAGGGGTCA TTAGTTTATA GGCATATAT GGAGTTCCGC GTTACATAAC
      AAGCTCGAGC GGGCTGTAC TAAATAGTGA TCAATAATTA TCATTAGTGA ATGCCCCAGT AATCAAGTAT CCGGTATATA CCTCAAGCCG CAATGTATTG
      thaI
      fnuDII/mvni
      bstUI
      bshI236I
      aciI maeIII
      bslI
      101 TTACGGTAA TGGCCCGCT GCGTACCGC CCAACGACC CCGCCCATTC ACGTCAATAA TGACGTATGT TCCCATAGTA AGCCCAATAG GGACTTTCCA
      AATGCCATTT ACCGGGCGA CCGACTCGCG GGTTCCTGG GCGGGGTAAC TGCAGTTATT ACTGCATACA AGGTATCAT TCGGTATC CCTGAAGGT
      maeII
      hnlII/acyI
      ahaII/bsaHI
      aatII
      201 TTACAGCTCA TGGGTGAGT ATTACGCTA AACTGCCAC TTGGCAGTAC ATCAAGTGA TCATATGCCA AGTACGCCG CTATTGAGT CAATGACGGT
      AACTGCAGT ACCACCTCA TAAATGCCAT TTACGGGTG AACCGTCATG TAGTTACAT AGTATACGCT TCATGCCGGG GATACTGCA GTTACTGCCA
      maeII
      hnlII/acyI
      ahaII/bsaHI
      aatII
      rsaI
      csp6I
      ndeI
      bglI
      201 TTACAGCTCA TGGGTGAGT ATTACGCTA AACTGCCAC TTGGCAGTAC ATCAAGTGA TCATATGCCA AGTACGCCG CTATTGAGT CAATGACGGT
      AACTGCAGT ACCACCTCA TAAATGCCAT TTACGGGTG AACCGTCATG TAGTTACAT AGTATACGCT TCATGCCGGG GATACTGCA GTTACTGCCA

```

FIG. 16A

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```

scrFI
nvaI
ecorII
acII
bglI dsav
sau96I bstNI
haeIII/palI
asuI apyI(dcm+)
301 AAATGGCCCG CTGCGATT TCCCGATAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACC TATTAGTCAT CGCTATTACC ATGCTGATCC
TTTACCGGCC GGACCGTAAT ACCGCTCATG TACTGGAATA CCTGAAAGG ATGAACCGTC ATGTAGATCC ATAATCAGTA CGGATAATGG TACCACTACG
nlaIII styI ncoI
dsal hphI acII
bsaJI sfaNI
301 AAATGGCCCG CTGCGATT TCCCGATAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACC TATTAGTCAT CGCTATTACC ATGCTGATCC
TTTACCGGCC GGACCGTAAT ACCGCTCATG TACTGGAATA CCTGAAAGG ATGAACCGTC ATGTAGATCC ATAATCAGTA CGGATAATGG TACCACTACG
maeII hinfI/acyI nlaIV
ahaiI/bsaHI hgiCI
aatII bafI
401 GGTGTTGCCA CTACATCAAT GCGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCACAGTC TCCACCCCAT TGACGTCAAT CGCAGTTTGT TTGCGACCA
CCAAACCGT CATGTAGTTA CCGGCACCTA TCGCCAACT GAGTGCCCT GAGTGCCCT AAAGTTTCAG AGGTGGGTA ACTGCAGTTA CCTCAACA CAACCGTGT
alul
estI
sacI
hgiJII
hgiAI/aspHI
ecII36II
bspI286
bsiHKA I
bafI
501 AAATCAACGG GACTTTCCAA AATGTGTA CAATCCGCC CCATTGACGC AAATGGCGG TAGCGGTGTA CGGTGGGAGG TCTATATAG CAGAGCTCGT
TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG GTTACCGCC TTTACCGCC ATCCGCACAT GCCACCGTCC AGATATATTC GTCTCGACCA
maeIII acII hgaI
acII csp6I mnlI
501 AAATCAACGG GACTTTCCAA AATGTGTA CAATCCGCC CCATTGACGC AAATGGCGG TAGCGGTGTA CGGTGGGAGG TCTATATAG CAGAGCTCGT
TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG GTTACCGCC TTTACCGCC ATCCGCACAT GCCACCGTCC AGATATATTC GTCTCGACCA

```

FIG. 16B

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```

        haeIII/palI
        mcrI
        eagI/xmaIII/ecI XI
        eaeI
        cfrI
        fnu4HI
        aciI
        thal
        fnuDII/mvnl
        sacII/sstII
        nspBII
        kspI scrFI
        dsal nciI
        bglI bali mspI
        sau3AI mnlI bstUI
        mboI/ndelI(dam-) hpaII
        dpnI(dam+) bsaJI dsav
        dpnI(dam-) bsh1236I
        alwI(dam-) aciI caulI
        sau96I
        avall
        asuI
        nlaIV
        scrFI
        nciI
        mspI
        hpaII
        dsav
        mboII
        bpuAI
        bbsI
        mnlI
        sau3AI gsuI/bpmI
        mboI/ndelI(dam-)
        dpnI(dam+) hgaI foki
        dpnI(dam-) ahaII/bsaHI
        601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCAGCCT GTTTGACCT GCATAGAGCA CACCGGACCC GTAGCTGCGA CAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGCTCGA GCGCCGCGC CTGGCJAGCT
        ANTCACTGG CAGTCTAGCG GACCTCTCGG GTAGCTGCGA CAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGCTCGA GCGCCGCGC CTGGCJAGCT
        ~Begin RNA

        tflI
        aciI
        thal hinfI
        fnuDII/mvnl
        bstUI
        bsh1236I
        701 TTGGNACCGG GATTCGCCGT GCCAGAGCTG ACGTAAGTAC CGCTATAGA GTCTATAGC CCACCCCTT GGCTTCGTTA GAAGCGCGCT ACAATTAAATA
        AACCTTGCGC CTAGGCGCA CGGTTCTCAC TGCATTTCATG CCGGATATCT CACATATCCG GCTGGCGGAA CCGAGCAAT CTGGCGCGA TGTTAATTAT
        ~sp6 promoter

        fnu4HI
        aciI
        thal
        fnuDII/mvnl tru9I
        bstUI mseI
        bsh1236I aseI/asnl/vsPI
        GGCTTCGTTA GAAGCGCGCT ACAATTAAATA
        CCGAGCAAT CTGGCGCGA TGTTAATTAT

```

FIG. 16C

[illegible]

FIG. 16D

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hgiJII
 bsp1286
 bayI
 banII
 scrFI
 mval nlaIV
 ecorII
 dsav
 bstNI
 bsajI
 apyl[dcmt] fokI
 1101 TTCATTCTG GCGTCCACC CATCTTGACC TTATCCAAGA TGCACACAGC ACTGGCAGTC TACCAACAGA TCTCACCAG TATGCTTCC AGAAGCTGA
 AAGTAAGGAC CCGAGTGG GTAGGACTG AATAGCTTCT ACTGGTCTG TGACCGTCTG ATGTTGTCT AGCAGTGGT ATACCGAAG TCTTTGCACT
 62 PheileProG lylLeuHisPr olleLeuThr LeuSerLysM elAspGlnTh rLeuAlaVal TyrGlnGlnI leLeuThrSe rMetProSer ArgAsnValile
 hphI
 mnlI
 sau3AI bsrI
 mboI/ndeII[dam-]
 dpnI[dam+]
 dpnII[dam-]
 alwI[dam-]
 bstYI/xhoII
 maeII
 sau96I
 avall
 asuI
 bsrI accI
 bstYI/palI
 pmlI
 sau3AI ecorII
 bstYI/xhoII
 gsuI/bpmI
 scrFI nclI mboI/ndeII[dam-]
 mval mspI dpnI[dam+]
 ecorII hpaII mboII maeII
 dsav dsav dpnII[dam-]
 bstNI mnlI alwI[dam-] bbrPI haeIII/palI fnu4HI
 apyl[dcmt] cauII mboII[dam-] haeI ddel aluI
 1201 TCCAAATATC CAACGACTG GAGAACCTCC GGGATCTTCT TCACGTGCTG GCTTCTCTA ACAGCTGCCA CTTGCCCTGG GCCAGTGGC TCGAGACCTT
 AGCTTTATAG GTTCTGAC CTCTGGAGC CCCTAGAAGA AGTGCACGAC CCGAAGAGAT TCTCGACCGT GAACGGGACC CCGTACCGG ACCTCTGGAA
 96 GlnlleSe rAsnAspLeu GluAsnLeuA rAspLeuLe uHisValleu AlaPheSerL ysSerCysH sLeuProTrp AlaSerGlyL euGluThrLeu
 scrFI ecorII
 mval scrFI
 ecorII mval aluI
 dsav ecorII
 bstNI dsav
 bsajI
 apyl[dcmt] apyl[dcmt] haeI
 bstNI bstNI
 bsajI bstXI apyl[dcmt] haeI
 apyl[dcmt] haeI
 sau96I
 avall
 sau96I
 aluI
 pvuII
 nspBII
 fnu4HI
 bbvI asuI
 1301 GGACAGCTG GGGGTGTC TGAAGCTTC AGCTACTCC ACAGAGGTGG TGGCCCTGAG CAGCTGCGC GGGTCTCTG AGGACATGCT GTGCGAGCTG
 CCTGTGGAC CCCCCAGG ACCTTGGAG TCGATGAGG TGTCTCCACC ACCGGACTC GTCCGAGCTC CCCAGAGCTC TCTGTACGA CACGTCGAC
 129 AspSerLeu GlyGlyVal euGluAlaSe rGlyTyrSer ThrGluValV alAlaLeuSe rArgLeuGln GlySerLeuG lnaPheMetLe uTrpGlnLeu

FIG. 16E

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```

scrFI      eam11051
mvaI       sau961
ecorII
dsav
bstNI
bsaJI
ddel apyI[dcn+] hphI
mnlI bsaJI aciI bstEII
CTGGAGTGG GACCCAGGCC CCAGTGGCTG TTTTGAGTGT GTACGGGTGG CACGGGTGG CCTGAACCTCC TGGGGGACC GTGAGTCTTC CTCTTCCCCC
1401 CTGGAGTGG GACCCAGGCC CCAGTGGCTG TTTTGAGTGT GTACGGGTGG CACGGGTGG CACGGGTGG GAGTCTGAGG ACCCCCTGG CAGTCAGAG GAGNAGGGGG
162 AspLeuSerP roGlyCysGI yValThrAsp LysThrHisT hrcysProPr oCysProAla ProGluLeuL euGlyGlyPr oSerValPhe LeuPheProPro
~Insertion of a gly
~START OF HUMAN IgG1 CH2CH3

sau96I
nlalV
mspI
hpall
scrFI
ncII
dsav
sau3AI avall
mboI/ndelI[dam-] nlalII
nlalII caulI mnlI nspl
rcal dplI[dam+] ddel nsphI
bspHI[dam-] asuI eco8II maelII
mnlI dplII[dam-] bsu36I/mstII/sauI
1501 CAAAGACCCAA GGACACCCCTC ATGATCTCCC GGACCCCTGA GGTACATGC GTGGTGGTGG ACCTGAGCCA CGAAGACCCCT GAGGTCAAGT TCNACTGGTA
GTTTGGGTG CTGTGGGAG TACTAGAGG CCTGGGGACT CCAGTGTACG CACCACCACC TGCCTCGGT CCTTCTGGGA CTCCAGTTCA AGTTGACCAT
196 LysProLy sAspThrLeu MetIleSera rgThrProGI uValThrCys ValValVala spValSerHI sGluAspPro GluValLysP heAsnTrpTyr

```

FIG. 16F

FIG. 16G

FIG. 16I

[illegible]

FIG. 16J

mau3AI
 mboI/ndeII(dam-)
 dpmI(dam+)
 dpmII(dam-)
 mnlI
 maml(dam-)
 beaBI(dam-)
 foki alvi(dam-) nlaIII
 2901 ACAGCATGAG GATGTTTC CATGATTGAA CAAGATGGAT TGCACGCAGG TTCTCCGCC CCTTGGGTGG AGAGGCTATT CGGCTATGAC TCGGCACAAC
 TGTCTACTC CTAGCAAGC GTACTACTT GTTCTACCTA AGTTCCTCC AGAGCCCGG CGAACCCACC TCTCCGATAA GCCGATACTG ACCCGTGTG
 bspI286
 bmyI
 bari
 mnlI
 bspHI
 hpaII
 mspI
 cfrI
 eaeI
 eagl/xmaIII/ecI XI
 mcrI
 haeIII/palI
 fnu4HI
 aciI
 3001 AGACAAATCGG CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGCGG CGCCCGCTTC TTTTGTCAA GACCGACCTG TCCGGTGGCC TGAATGAACT
 TCTGTTAGCC GACGAGACTA CGCGCGCACA AGCCCGACAG TCGCGTCCCC GCGGCCCAAG AAAACAGTT CTGGCTGGAC AGCCACCGG ACTTACTTGA
 hlnPI
 hhaI/cfoI
 nlaIV
 nari scrFI
 kasi nciI
 hinII/acyI
 hgiCI mspI
 haeII hpaII
 bani dsav
 hlnPI ahaII/bsaHI
 hhaI/cfoI caulI
 drdI
 bsaHI
 hpaII
 mspI
 bni
 hgiCI
 nlaIV
 bmyI
 bspI286
 hgiAI/aspHI
 bspI286
 aluI
 pvuII
 fnu4HI
 bbvI bslHRAI
 hinPI bmyI
 hhaI/cfoI
 mstI nspBII
 aviII/fspI
 taqI
 tthIII/aspI
 maeII
 eco37I
 fnu4HI
 bsrI bbvI
 3101 GCAGGACGAG GCAGCGCGC ZATGCTGGCT GCCCAGCAGC GCGTTCCTT GCTGACGTT GTCACTCAAG CGGGAAGGA CTGGCTGCTA
 CGTCTGCTC GTTCGCGCGG ATAGCACCGA CCGGTGCTGC CCGCAAGGAA CGGCTGCACA CGAGCTGCAA CAGTGACTTC GCCTTCCCT GACCGACGAT
 mnlI
 hhaI/cfoI
 bbvI aciI
 fnu4HI
 hinPI
 bshI236I
 bstUI
 fnuDII/mvni
 thai
 fnu4HI
 mnlI
 hhaI/cfoI
 bbvI aciI
 fnu4HI
 hinPI
 haeIII/palI
 mscI/balI
 haeI
 eaeI
 cfrI
 mstI nspBII
 aviII/fspI
 taqI
 tthIII/aspI
 maeII
 eco37I
 fnu4HI
 bsrI bbvI
 CGTCTGCTC GTTCGCGCGG ATAGCACCGA CCGGTGCTGC CCGCAAGGAA CGGCTGCACA CGAGCTGCAA CAGTGACTTC GCCTTCCCT GACCGACGAT

FIG. 16K

3201 TTGGGGCAAG TCGCGGGCA CGATCTCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGGGGCTG CATACGCTTG
 AACCGCTTC ACCGCCCCGT CCTAGAGGAC AGTAGAGTGG AACGAGGACG GCTCTTTCAT AGGTAGTACC GACTACGTTA CCGCCCGGAC GTATCGGAAC
 sau3AI mboI/ndeII{dam-} fnu4HI sau3AI mboI/ndeII{dam-}
 nciI dpnI{dam+} fnu4HI dpnI{dam+}
 mspI hpaII dsaV dpnII{dam-} aciI alvi{dam-}
 cauII bstYI/xhoII fnu4HI aciI bbvI
 bsaJI alvi{dam-} hphI nlaIII sfaNI
 3301 TTGGGGCAAG TCGCGGGCA CGATCTCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGGGGCTG CATACGCTTG
 AACCGCTTC ACCGCCCCGT CCTAGAGGAC AGTAGAGTGG AACGAGGACG GCTCTTTCAT AGGTAGTACC GACTACGTTA CCGCCCGGAC GTATCGGAAC
 sau3AI mboI/ndeII{dam-} foki
 rsaI csp6I
 bsaAI hgiAI/asphi
 bspI286 mspI dpnI{dam+} sau3AI dpnI{dam+} sapi
 taqI bsiHKAi hpaII dpnII{dam-} mboI/ndeII{dam-} mspI dpnI{dam+} mboI
 bsaJI alvi{dam-} hphI nlaIII sfaNI foki cfr10I taqI{dam-} dpnII{dam-} earI/ksp632I
 3301 ATCCGCTAC CTGCGCTAC GACCAACATCG CGAACATCG GCACGTACTC GGATGGAGC CGGTCTTGTG GATCAGGATG ATCTGACGA
 TAGGCGGATG GACGGGTAG CTGGTGGTTC GCTTGTAGC GTAGTGGCT CGTGCATGAG CCTACTCTTC GCCAGAACAG CTAGTCTCTAC TAGACCTGCT
 mspI hpaII bspHI taqI
 3401 AGAGCATCAG GCGCTCGCG CAGCGGAACT GTTCCGCCAG CTCAGGCCG CGATGCCGA CCGGAGGAT CTGCTGCTGA CCGATGCGGA TCGCTGCTTG
 TCTGCTAGTC CCGAGCGCG GTGGCTTGA CAGCGGTTC GAGTTCGCG CGTACGGCT GCCGCTCTA GAGCAGCACT GGGTACCGCT ACCGAGGAAAC
 sphi
 nspl
 nspl
 hinPI hhaI/cfoI
 thal
 fnuDII/mvni scrFI fnuDII/mvni mboI/ndeII{dam-} sau3AI
 bstUI mvaI dpnI{dam+} dpnI{dam+} styI
 hgiJII ecorII bsh1236I dpnII{dam-} ncoI
 bspI286 dsaV hinPI nlaIII bstVI/xhoII dsaI
 bsaJI alvi{dam-} bsaJI sfaNI
 mniI
 3401 AGAGCATCAG GCGCTCGCG CAGCGGAACT GTTCCGCCAG CTCAGGCCG CGATGCCGA CCGGAGGAT CTGCTGCTGA CCGATGCGGA TCGCTGCTTG
 TCTGCTAGTC CCGAGCGCG GTGGCTTGA CAGCGGTTC GAGTTCGCG CGTACGGCT GCCGCTCTA GAGCAGCACT GGGTACCGCT ACCGAGGAAAC
 sfaNI
 apyI{dcm+} bshII
 banII hhaI/cfoI
 bsaJI alvi{dam-} bsaJI sfaNI

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FIG. 16L

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[illegible]

FIG. 16M

[illegible]

acil
 thai
 fnuDII/mvni
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

mspi
 hpall
 acil
 fnu4HI
 GCCGCCGGAC GAACTAAACC TGACTACGGC ATCTCTGCC CTTCCTCGT
 CGCGGGCTG CTTGATTTCG ACTGATGCCG TAGACACGGG GAAGAAGCGA CCATGCTCT CGCGAAACA

hinPI
 mnII hhai/cfoI
 rsal
 csp6I
 eco47III
 mboII
 sfaNI
 balI
 bsaNI
 bsaJI
 bstNI
 sau96I
 nlaIV haeIII/palI
 avalI eaeI
 asul cfrI bsp1286
 ppunI mspi apyI(dcm+)
 nlaIV hpall bayI
 eco109I/draII bani
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfrI acil
 TTTGATTGG TCACCACGGC CGAGTTTCCG
 AACATAACC AGTGGTGCCG GCTCAAGGCC

hinPI mspi
 hhai/cfoI
 hpall
 fnuDII/mvni
 bstUI bsaNI
 bshI236I
 fnu4HI
 acil bcoI
 nlaIII acil bali
 nlaIII
 mboII
 bpuAI
 bbaI
 nlaIII
 CGCGACCCCG GCCAGGGCAC CTGTCTTACG AGTTGCATGA TAAAGAGAC AGTCATAAGT GCGCGGACGA TAGTCATGCC CGCGCCAC CGGAGGAGC
 GCCTGGGC CGCTCCCGTG GACAGATGC TCAACGTACT ATTTCTCTG TCAGTATTCA CGCCGCTCT ATCAGTACGG GCGGGGTC GCCTCTCTCG

acil
 bstUI
 sacII/sstII
 haelII/palI bshI236I
 mcrI
 dsal
 kspi
 dsal
 bsajI
 hphi eagI/xmaII/eciXI
 maeIII eaeI bsajI
 bateII cfr

FIG. 16P

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```

fnu4HI      acil
haeIII/palI  fnu4HI
mcrI        thal
eagI/xmaII/eklXI  fnuDII/mvnl
eaeI        bstUI
notI        hinPI
fnu4HI      bstUI
acil        bsh1236I
mcrI bsrBI acil      hhaI/cfoI hhaI/cfoI
sfaNI taqI cfrI sfaNI  hhaI/cfoI hhaI/cfoI
4601 TCACTGGGT GAAGGCTTC AGGGGCTCG GTGAGCGGC CGCATCAAG CAACATAGT AGCGCCCTG TAGCGCGCA TTAAGCGCG CGGGTGTGCT
ACTGACCCAA CTTCGAGAG TTCCGTAGC CAGCTCGCG CGGTAGTTC GTTGTATCA TCGCGGGAC ATCGCGCGT AATCGCGCC GCCCACACCA
      *delta 3
      *H13 ori

fnu4HI      hinPI
hinPI      hhaI/cfoI
hhaI/cfoI  hhaI/cfoI
thal        hhaI/cfoI
fnuDII/mvnl  hhaI/cfoI
bstUI      hhaI/cfoI
bsh1236I   hhaI/cfoI
maeIII bbvI maeIII  hhaI/cfoI hhaI/cfoI
4701 GGTACGGC AGGTGACCG CTACACTGC CAGCGCCCTA CGCGCCCTC CTTCGCTT CTTCCTTC TTCTCGCA CGTTCGCGG CTTTCCCGT
CCAAATGGCG TCGCACTGC GATGTGACG GTCGCGCAT CGCGGGCGAG GNAAGCGAA GAAGGAGGT GNAAGCGCC GAAAGGGCA

nlaIV      maeII haeIII/palI
hgiIII     draIII sau96I
bap1286    bsaAI asuI
bavI       hphI
banII      bphI
alul       bphI
4801 CAAGCTCTAA ATCGGGGCT CCCTTTAGG TTCCGATT TA GCTTTAGC GCACCTGCAC CCCAAAAAC TTGATTTGG TGTGTTCA CGTAGTGGC
GTTGAGATT TAGCCCGA GGAATCC AGGCTAAT CAGCAATCC CGTGGAGTC GGTTTTTT ACTAACC ACTACCACT GCATCACCC

nlaIV      maeII pleI
hgiIII     drdI hinfI maeII pleI
bap1286    drdI hinfI maeII hinfI
bavI       hinfI
banII      hinfI
4901 CATGGCCCTG ATAGAGGTT TTTCGCCCTT TCAGGTGGA GTCCACCTTC TTTAATAGT GACTCTGT CCAACTGGA ACAACTCA ACCCTATCTC
GTAGCGGAC TATCTGCCA AAAGCGGAA ACTGCACT CAGGTGCAAG AAATTATC CTGAGAACA GGTTCACCT TGTGTGACT TGGATAGAG

```

FIG. 16Q

FIG. 16R

FIG. 16S

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```

mspi          bglI
hpaiI         sau96I
scrFI         haeIII/paiI
              hinfI asuI mspI
              hhaI/cfoI hpaiI
5901 AACTACTTAC TCTAGCTTC CGGCACACAT TAATAGACTG GATAGAGCG CATANAATTG CAGGACCACT TCTGGCTCG GCGCTTCGG CTGGCTGGTT
      TTGATCAATG AGATCGAAGG GCGGTGGTA ATTATCTGAC CTACTCCG CTATTCAAC GTCTGTGTA AGACCGGCGC CCGAAGGCC CACCGACCAA

              acII
              thal
              fnuDII/mvni sau96I
              bstUI asuI
              bsmAI fnu4HI nlaIV
              bsaI bsh1236I bbvI bsrI haeIII/paiI mnlI
6001 TATTGCTGAT AATCTGGAG CCGGTGAGCG TGGGTCTCGG GGTATCATTTG CAGCACTGG CCGCATGCT AAGCCTCCC GTATGCTAGT TATCTACAG
      ATACCACTA TTACAGCTC GGCACCTCG ACCCAGACCG CCATAGTAAC GTCTGACCC CGTCTACCA TTCGGAGCG CATAGCATCA ATAGATGTCC
              ddeI
              sau3AI nlaIV
              mboI/ndeII[dam-] mnlI
              dpnI[dam+] hgiCI tru9I
              dpnI[dam-] bsaI mseI maeII
              foki
              pleI
              hinfI
6101 ACGGGGAGTC AGGCAACTAT GATGAACCA AATAGACAGA TCGTGCAGAT AGGTGCCTCA CTGATTAGC ATTGTAAC GTGAGACCAA GTTTACTCAT
      TGGCCCTCAG TCGCTTGATA CCTACTTCT AGGCACTCTA TCCACGGAGT GACTAATTCG TAACCATTTA CAGTCTGGTT CAATGAGTA

              hphI
              rmaI sau3AI
              sau3AI mboI/ndeII[dam-]
              mboI/ndeII[dam-]
              dpnI[dam+] dpnI[dam+]
              tru9I dpnI[dam-] dpnI[dam-]
              ahaIII/draI maeI alwI[dam-]
              tru9I bstYI/xhoII bstYI/xhoII nlaIII
              maeI maeI alwI[dam-] mboII[dam-] rcaI
              ahaIII/draI tru9I maeI tru9I
              maeI maeI alwI[dam-] mboII[dam-] bspHI
6201 ATATACTTTA GATTGATTTA AACTTCATT TTTAATTTAA AGGATCTAG GTGAGATCC TTTTGTATA TCTCATGACC AAATCCCTT AACGTGAGTT
      TATATCAAT CTACTAAT TTGAGTAA AATTAATTTT TCTTAGATC CACTTCTAGG AAATCTATT AGACTACTGG TTTTAGGAA TTGCACCTAA

```

FIG. 16T

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```

sau3AI
mboI/ndelII(dam-)
dpmI(dam+) sau3AI
dpmII(dam-) mboI/ndelII(dam-)
bstYI/xhoII dpmI(dam+) fnuDII/mvnI
sau3AI alwI(dam-) dpmII(dam-) bstUI
mboI/ndelII(dam-) alwI(dam-) bsh1236I
dpmI(dam+) mboII(dam-) hinPI fnu4HI
dpmII(dam-) bstYI/xhoII hhaI/cfoI bbvI
6301 TTGTTCCAC TGACGGTCAG ACCCGTAGA AAGATCAAA GGATCTTCTT GAGATCTTCTT TTTCTGCGC GTAATCTGCT GCTTGCAAC AAAAAACCA
AAGCAGGTG ACTGGCAGTC TGGGCATCT TTCTAGTTT CCTAGAGAA CTCTAGGAA AAGAGCGCG CATTAGCCA CGAAGGTTG TTTTTCGT
dclI hgaI
6401 CCGCTACAG CCGTGGTTC TTTGCGGAT CAAGAGCTAC CAATCTTTT TCCGAGGTA ACTGGCTTCA GCAGAGGCA GATACCAAT ACTGCTGTC
GGGATGTC GCCACCAAC AAGCGCTA GTTCTCGATG GTTGAGAAA AGCTTCCAT TGACCGAAGT CGTCTCCGT CTATGTTTA TGACAGGAAG
acII nsp8II hpaII aluI
acII nsp8II hpaII aluI maelII eco57I hhaI/cfoI rmaI
6501 TAGGTAGCC GTAGTAGCC CACCACTTCA AGAATCTGT AGCACTGCTT ACATAGCTCG CTCTCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGGA
ATCAGATGG CATCAATCG GTGGCAAGT TCTTGAGACA TCGTGGCGGA TGTATGGAGC GAGACGATTA GGACAATGT CACCGACGAC GGTCAAGCT
haeIII/palI
bsII haeI
6601 TAACTGCTGT CTTACCGGT TGAAGTCAAG ACCATAGTTA CCGGATAAGC CCGACGCTC GGGCTGAACG GGGGGTTGCT GCACACAGCC CAGCTTGGAG
ATTACGACA GAATGGCCA ACTGAGTTC TGCTATCAAT GGCCTATTCC GCGTGGCAG CCGGACTTGC CCCCCAAGCA GGTGTGTGG GTCGAAGCTC
scrPI nclI
nsp8II
mspI fnu4HI
hpaII bbvI
dsav pleI bsaXI hinPI acII
cauII hinfI maelII hhaI/cfoI
hgaI/asphI
bsp1286
bsiHKA I
bayI
apaLI/snoI
alw44I/snoI
aluI

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FIG. 16U

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6701 CGAAGCACT ACACCGAAT GAGATACCTA CAGCGTGAGC ATTGAGAAAG CGCCACGCTT CCGGAAGGGA GAAAGCGGGA CAGGTATCCG GTAAGCGGCA
GTTTCTGGA TGTGCTTGA CTCTATGGAT GTGGCACTCG TAACTCTTTC GCGGTGCGAA GGGCTTCCCT GTTCCGCT GTCCATAGGC CATTCGCCGT

        ddeI      srfI      hinfI      hhaI/cfoI      hpaII      fnu4HI
        mspI      bsaBI      acII      bsaBI      acII
        scrFI      mvaI      ecorII      mvaI      dsav      ecorII      bstNI      bsaJI      dsav      bstNI
        hinfI      mnlI      hhaI/cfoI      aluI      apyI(dcm+)      apyI(dcm+)      mnlI      drdI      hgaI      taqI
6801 GCGTGGAC AGGAGGCGC ACGAGGAGC TTCCAGCGG AAGCGCTGG TATCTTTATA GTCTGTGCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT
CCGAGCCTTG TCTCTGCGG TCTCTGCTCG TCTCTGCTCG AAGTCCCTT TTTCCGAC ATAGAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA

        aluI      nlaIV      acII      pvuII      hspBII
        scrFI      mvaI      ecorII      mvaI      dsav      ecorII      bstNI      bsaJI      dsav      bstNI
        hinfI      mnlI      hhaI/cfoI      aluI      apyI(dcm+)      apyI(dcm+)      mnlI      drdI      hgaI      taqI
6901 TTTGTGATC TCGTCAGGG GCGGAGCCT ATGGAAAAC GCGAGCTGGC ACCACAGCTT TCCGACTGG AAGCGGGA GTGAGCGCA CCGAATTAT
AACAATAGC AGCACTGCG CCGCTTGG TACTTTTG CCGTCGACCG TCTGTGCA TCGGTGACC AGGGCTGACC TTTCCGCT CACTCGCTT CCGTTATTA

        aluI      nlaIV      acII      pvuII      hspBII
        scrFI      mvaI      ecorII      mvaI      dsav      ecorII      bstNI      bsaJI      dsav      bstNI
        hinfI      mnlI      hhaI/cfoI      aluI      apyI(dcm+)      apyI(dcm+)      mnlI      drdI      hgaI      taqI
7001 GTGACTTACC TCACTCATTA GGCACCCGAG CTTTACACT TTATGCTTC GCGTCGTATG TTGTGTGGA TTGTGAGCGG ATAACAATT CACACAGGAA
CACTCAATGG AGTGAGTAAT CCGTGGGCTC CGAATGTGA ATAGCAAGG CCGAGCATAC AACACACCTT AACACTGCG TATTGTTAA GTGTGTCTT

        mnlI      ma III      hgaI      hpaII      mspI      acII      bsrBI
        scrFI      mvaI      ecorII      mvaI      dsav      ecorII      bstNI      bsaJI      dsav      bstNI
        hinfI      mnlI      hhaI/cfoI      aluI      apyI(dcm+)      apyI(dcm+)      mnlI      drdI      hgaI      taqI
7101 ACAGCTATGA CCAATGATTAC GAATTA TGTGATACT GGTACTATAG CTTAAT

        aluI      nlaIII      asp700
        scrFI      mvaI      ecorII      mvaI      dsav      ecorII      bstNI      bsaJI      dsav      bstNI
        hinfI      mnlI      hhaI/cfoI      aluI      apyI(dcm+)      apyI(dcm+)      mnlI      drdI      hgaI      taqI

```

FIG. 16V

>length: 7127

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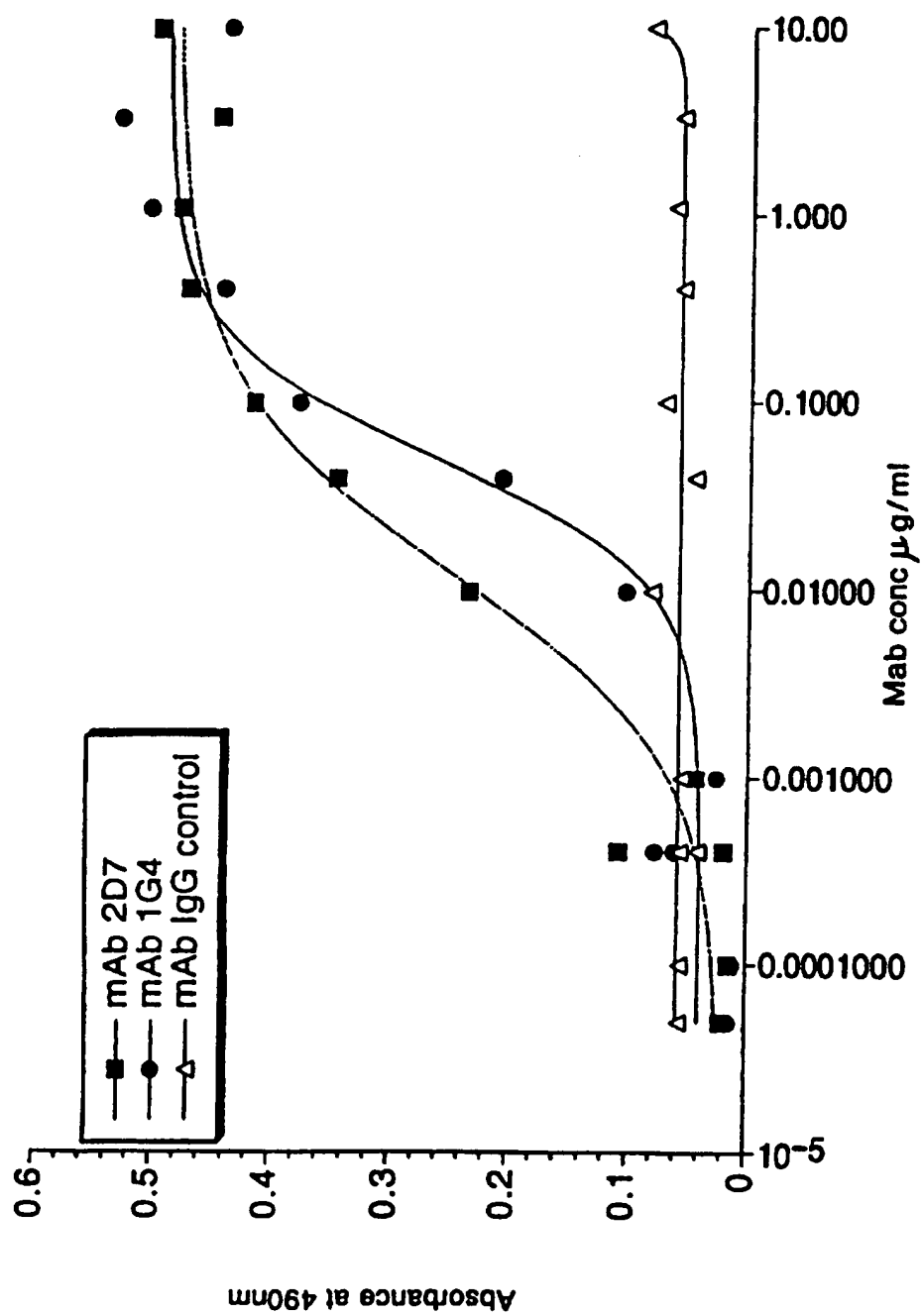


FIG. 17

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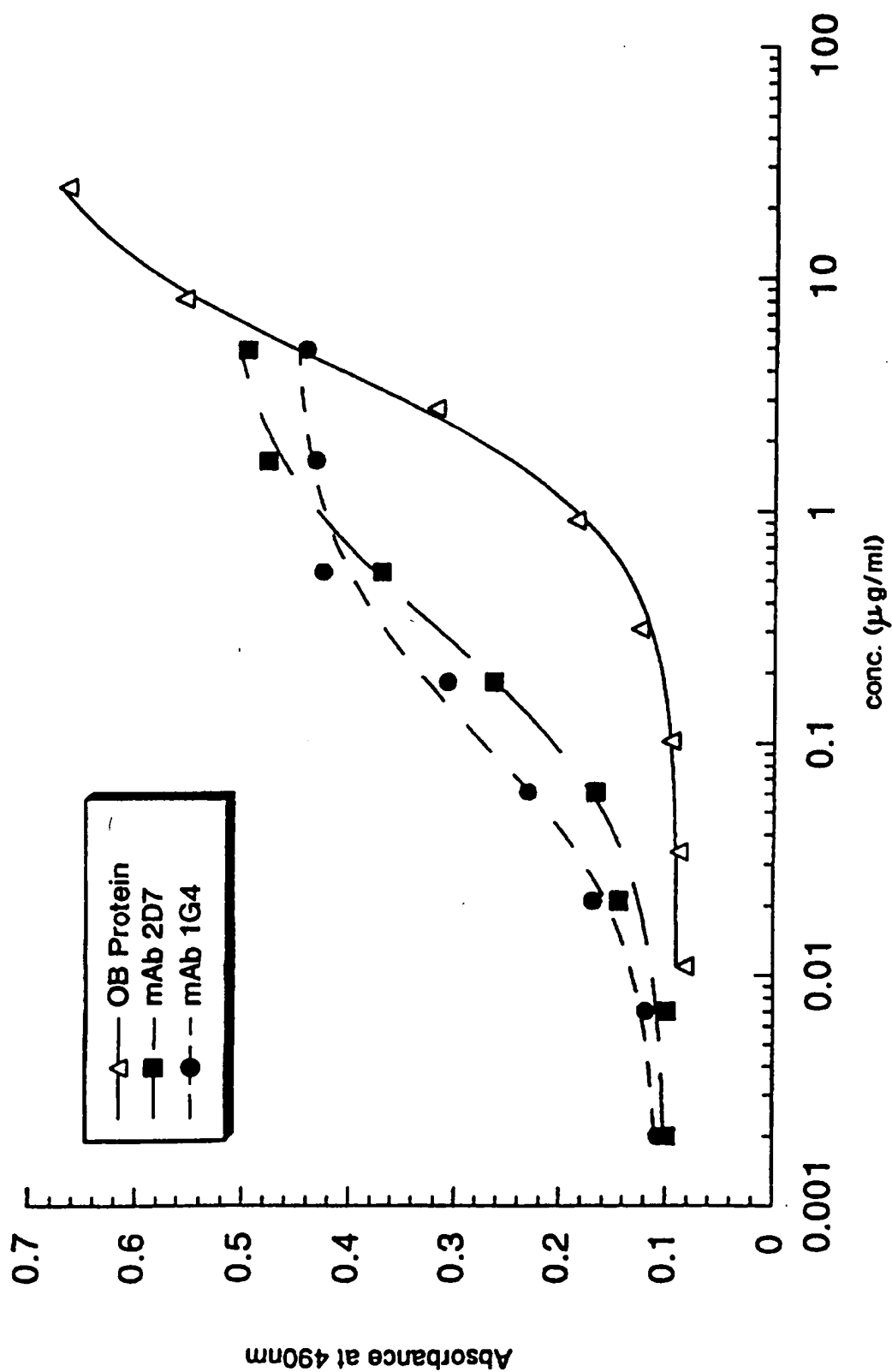


FIG. 18

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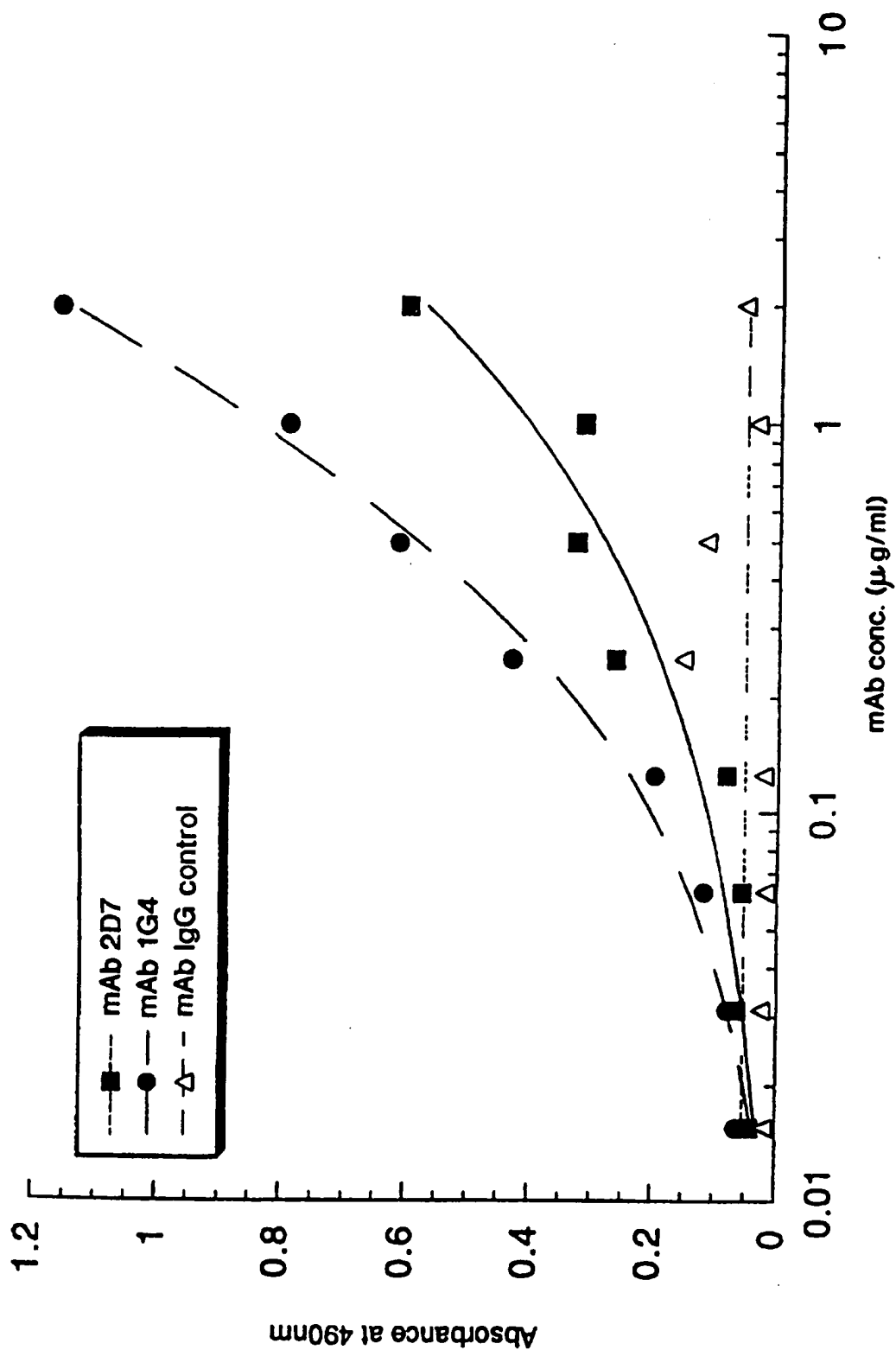


FIG. 19

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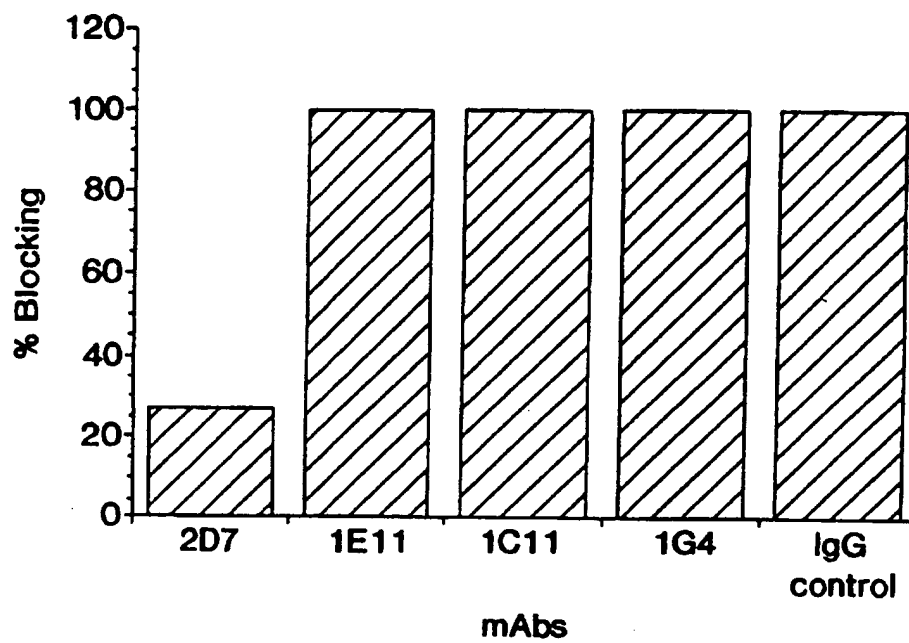


FIG. 20A

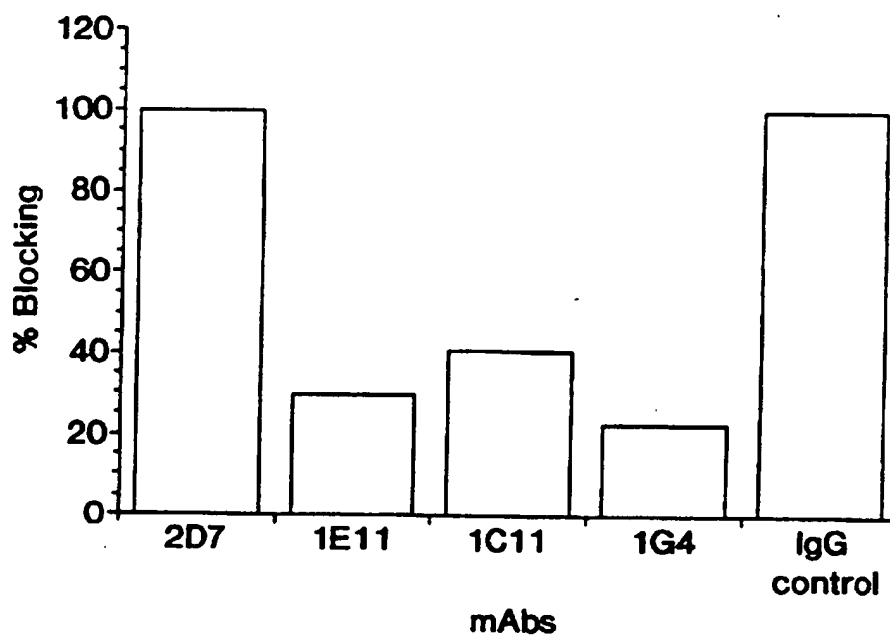


FIG. 20B

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1	M	I	C	Q	K	F	C	V	V	L	L	H	W	E	F	I	Y	V	I	T	A	F	N	L	S	Y	P	I	T	P	W	R	F	K	L	S	C	M	P	P	N	S	T	Y	D	Y	F	L	L	P
1	M	M	C	Q	K	F	Y	V	V	L	L	H	W	E	F	L	Y	V	I	A	A	L	N	L	A	Y	P	I	S	P	W	K	F	K	L	F	C	G	P	P	N	T	T	D	D	S	F	L	S	P
51	A	G	L	S	K	N	T	S	N	S	N	G	H	Y	E	T	A	V	E	P	K	F	N	S	S	G	T	H	F	S	N	L	S	K	T	T	F	H	C	C	F	R	S	E	Q	D	R	N	C	S
51	A	G	A	P	N	N	A	S	A	L	K	G	A	S	E	A	I	V	E	A	K	F	N	S	S	G	I	Y	V	P	E	L	S	K	T	V	F	H	C	C	F	G	N	E	Q	G	O	N	C	S
101	L	C	A	D	N	I	E	G	K	T	F	V	S	T	V	N	S	L	V	F	Q	I	D	A	N	W	N	I	Q	C	W	L	K	G	D	L	K	L	F	I	C	Y	V	E	S	L	F	K	N	
101	A	L	T	D	N	T	E	G	K	T	L	A	S	V	V	K	A	S	V	F	R	Q	L	G	V	N	W	D	I	E	C	W	M	K	G	D	L	T	L	F	I	C	H	M	E	P	L	P	K	N
151	L	F	R	N	Y	N	Y	K	V	H	L	L	Y	V	L	P	E	V	L	E	D	S	P	L	V	P	Q	K	G	S	F	O	M	V	H	C	N	C	S	V	H	E	C	C	E	C	L	V	P	V
151	P	F	K	N	Y	D	S	K	V	H	L	L	Y	D	L	P	E	V	I	D	S	P	L	P	P	L	K	D	S	F	O	T	V	Q	C	N	C	S	L	R	G	-	C	E	C	H	V	P	V	
201	P	T	A	K	L	N	D	T	L	L	M	C	L	K	I	T	S	G	G	V	I	F	Q	S	P	L	M	S	V	Q	P	I	N	M	V	K	P	D	P	P	L	G	L	H	M	E	I	T	D	D
200	P	R	A	K	L	N	Y	A	L	L	M	Y	L	E	I	T	S	A	G	V	S	F	Q	S	P	L	M	S	L	Q	P	M	L	V	V	K	P	D	P	P	L	G	L	H	M	E	V	T	D	D
251	G	N	L	K	I	S	W	S	S	P	P	L	V	P	F	P	L	Q	Y	Q	V	K	Y	S	E	N	S	T	T	V	I	R	E	A	D	K	I	V	S	A	T	S	L	L	V	D	S	I	L	P
250	G	N	L	K	I	S	W	D	S	Q	T	M	A	P	F	P	L	Q	Y	Q	V	K	Y	L	E	N	S	-	T	I	V	R	E	A	E	I	V	S	A	T	S	L	L	V	D	S	V	L	P	
301	G	S	S	Y	E	V	Q	V	R	G	K	R	L	D	G	P	G	I	W	S	D	W	S	T	P	R	V	F	T	T	Q	D	V	I	Y	F	P	P	K	I	L	T	S	V	G	S	N	V	S	F
299	G	S	S	Y	E	V	Q	V	R	S	K	R	L	D	G	S	G	V	W	S	D	W	S	S	P	Q	V	F	T	T	Q	D	V	V	Y	F	P	P	K	I	L	T	S	V	G	S	N	A	S	F

FIG. 21A

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351 H C I Y K K E N K I I V P S K E I V W W M N L A E K I P Q S Q Y D V V S D H V S K V T F F N L N E T K
 349 H C I Y K N E N Q I I S S K Q I V W W R N L A E K I P E I Q Y S I V S D R R V S K V T F S N L K A T R

401 P R G K F T Y D A V Y C C N E H E C H H R Y A E L Y V I D V N I N I S C E T D G Y L T K M T C R W S
 399 P R G K F T Y D A V Y C C N E Q A C H H R Y A E L Y V I D V N I N I S C E T D G Y L T K M T C R W S

451 T S T I Q S L A E S T L Q L R Y H R S S L Y C S D I P S I H P I S E P K D C Y L Q S D G F Y E C I F
 449 P S T I Q S L V G S T V Q L R Y H R R S L Y C P D S P S I H P T S E P K N C V L O R D G F Y E C V F

501 Q P I F L L S G Y T M W I R I N H S L G S L D S P P T C V L P D S V V K P L P P S S V K A E I T I N
 499 Q P I F L L S G Y T M W I R I N H S L G S L D S P P T C V L P D S V V K P L P P S N V K A E I T V N

551 I G L L K I S W E K P V F P E N N L Q F Q I R Y G L S G K E V Q W K M Y E V Y D A K S K S V S L P V
 549 T G L L K V S W E K P V F P E N N L Q F Q I R Y G L S G K E I Q W K T H E V F D A K S K S A S L L V

601 P D L C A V Y A V Q V R C K R L D G L G Y W S N W S N P A Y T V V M D I K V P M R G P E F W R I N
 599 S D L C A V Y V Q V R C R R L D G L G Y W S N W S S P A Y T L V M D V K V P M R G P E F W R K M D

651 G D T M K K E K N V T L L W K P L M K N D S L C S V Q R Y V I N H H T S C N G T W S E D V G N H T K
 649 G D V T K K E R N V T L L W K P L T K N D S L C S V R R Y V V K H R T A H N G T W S E D V G N R T N

FIG. 21B

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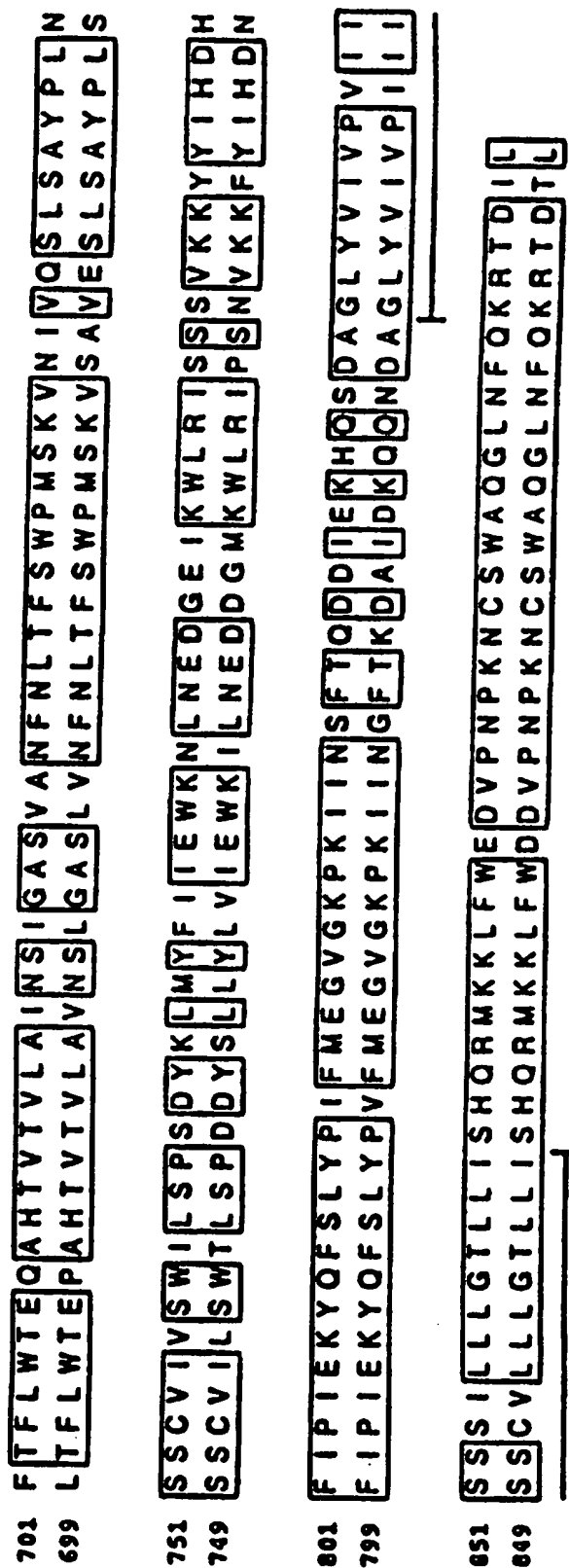
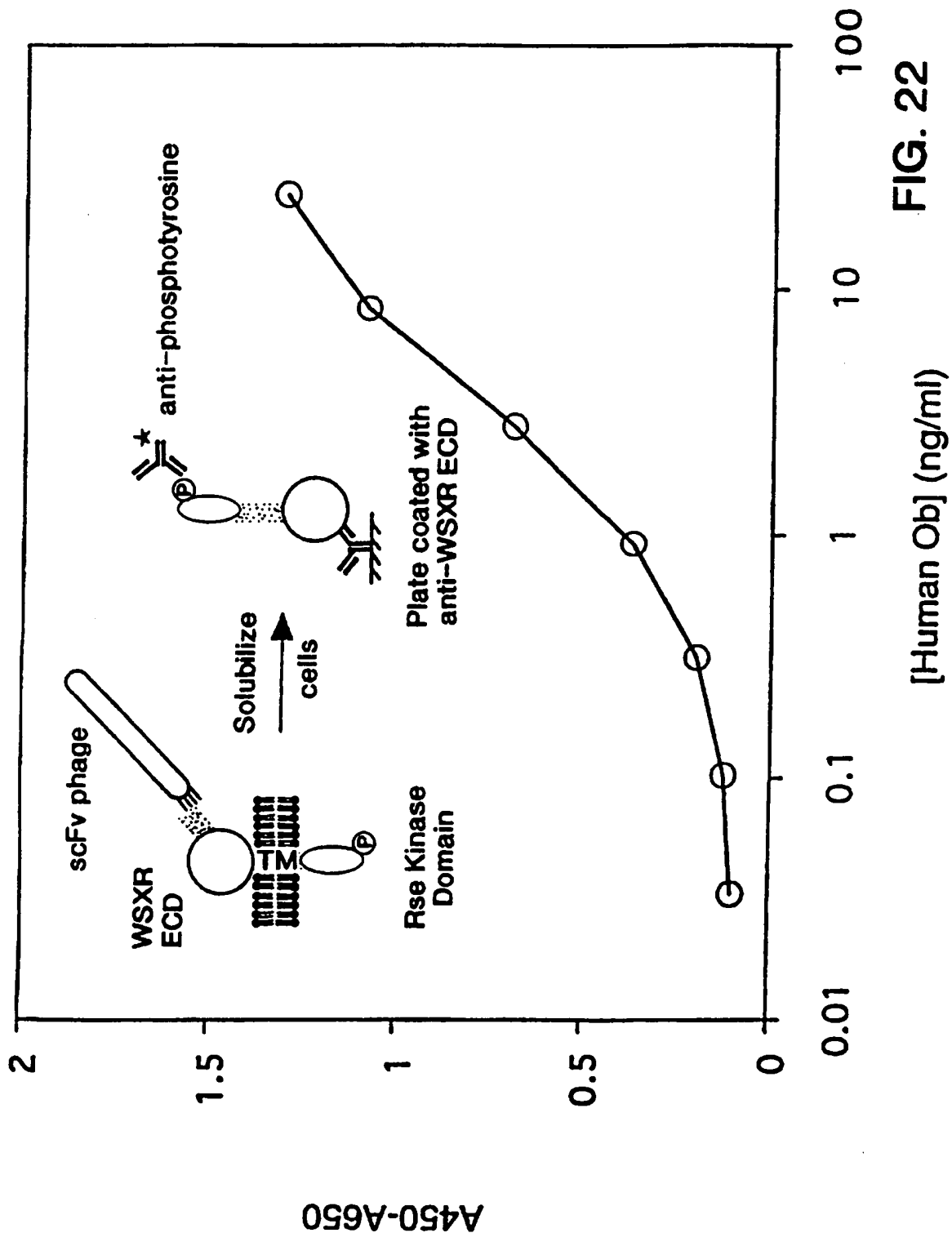


FIG. 21C



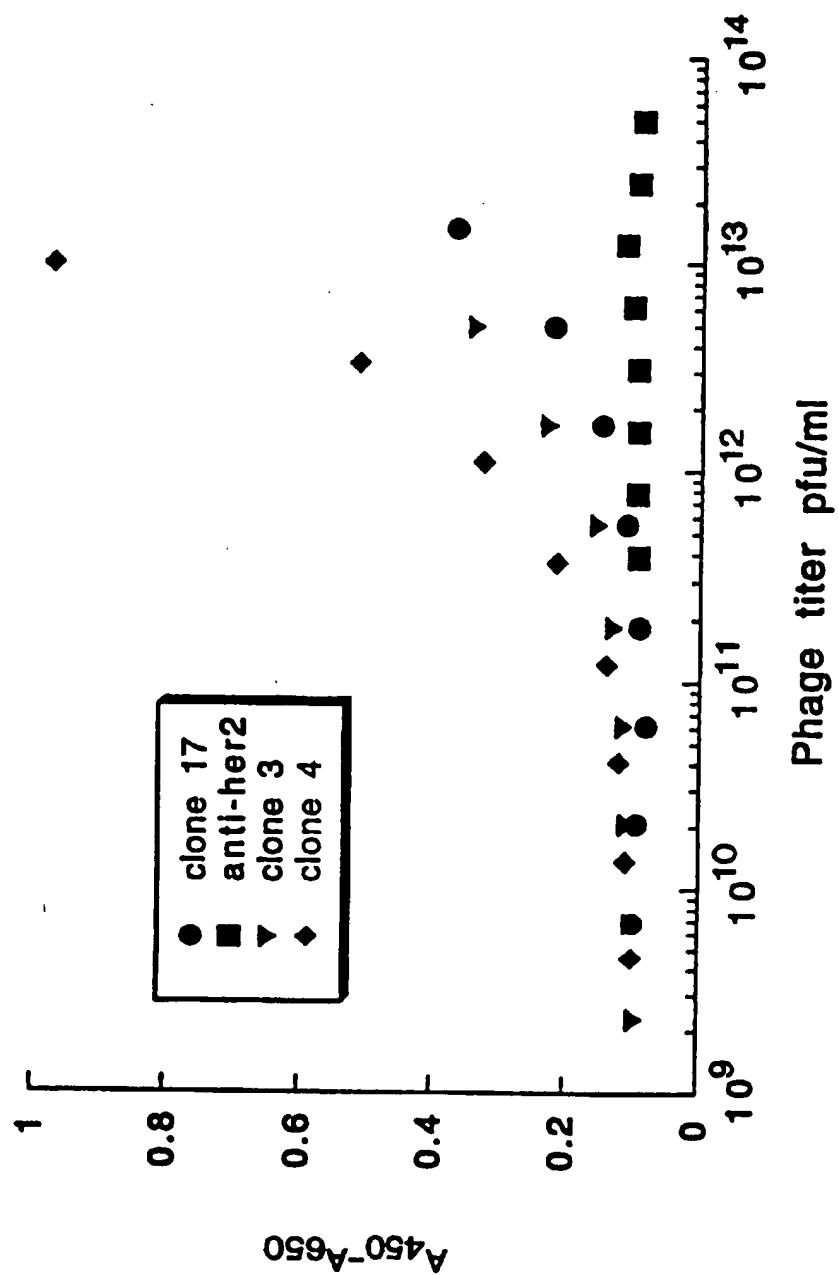


FIG. 23

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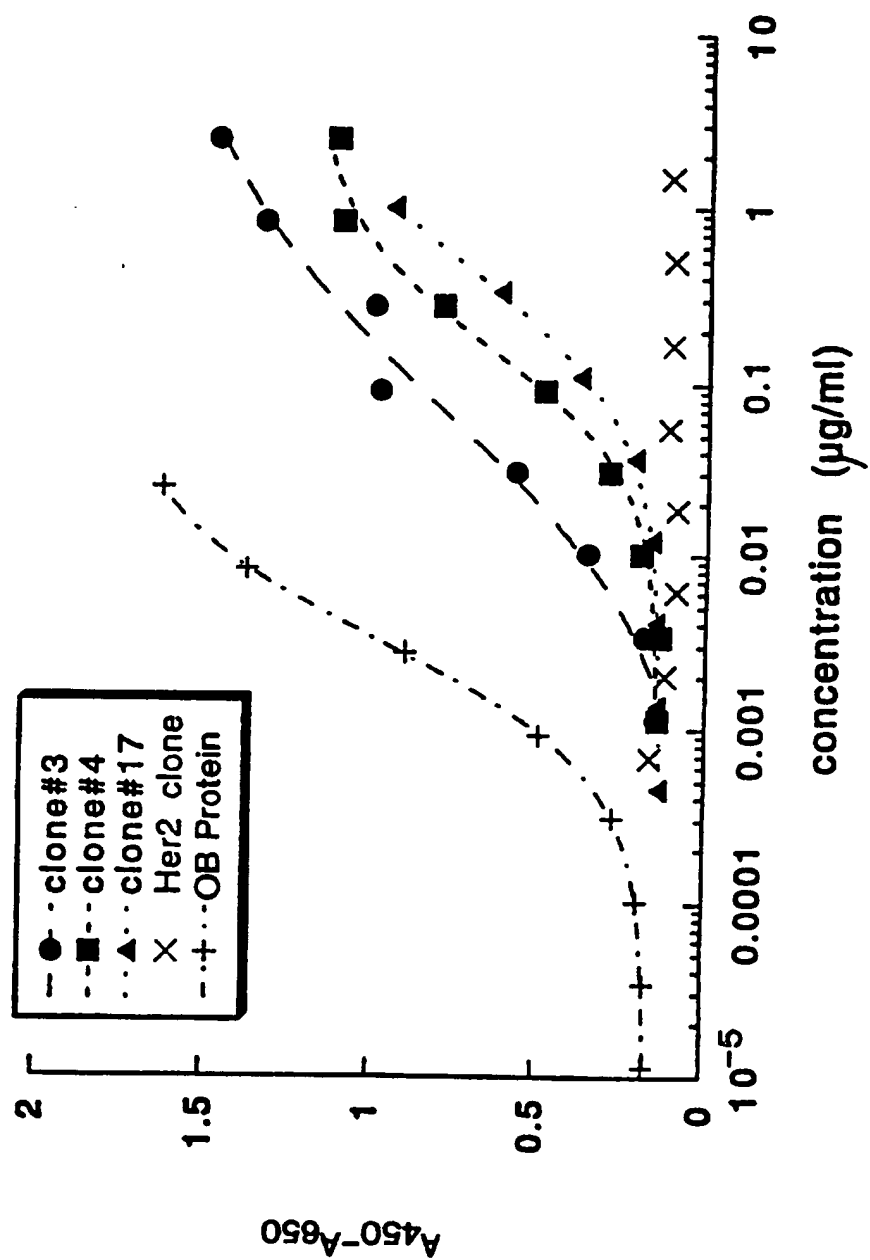


FIG. 24

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17.scfv 1 QVRLQQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSG
 3.scfv 1 EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMYWVRQAPGQGLEWMGH
 4.scfv 1 EVQLVQSGAEVKKPGESLKISCGSGFTFSSYKMNWVRQAPGKGLEWMGG
 CDR H1
 17.scfv 51 MTWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREP
 3.scfv 51 INPNSGGTNYAOKFOGRVTMTRDTSIGTAYMELSRSSDDTAVYYCARDR
 4.scfv 51 ILPIFGTANYAOKFOGRVTITADESTSTAYMELSSLRSEDVAVYYCARDR
 CDR H2
 17.scfv 101 HNTDA-----FDIWGRGTLVTVSSGGGGPGGGGSGGGGSDVVMTQSP
 3.scfv 101 YYGSSAYHRGSYYMDVWGRGTLVTVSSGGGGTGGGGSGGGGS-SELTQDP
 4.scfv 101 VVVPATSLRGG--MDVWGQGTTVTVSSGGGGSGGGGSGGGGSQSVLTQPA
 CDR H3
 17.scfv 143 SFLSAFVGDTITITCRASQ---GIYNYLAWYQOKPGKAPKLLIYAASTLO
 3.scfv 150 A-VSVALGQTVRITCOGDS--LRSY-YASWYQOKPGQAPVLVIYGKNRP
 4.scfv 149 S-VSGSPGQSITISCTGTSSDVGGYNYVSWYQOHGKAPKLMYEGSKRP
 CDR L1 CDR L2
 17.scfv 190 SGVPSRFSGSGSGTEFTLTISSLQPEDFGTYICQOLI--SYPLTFGGGTK
 3.scfv 196 SGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTK
 4.scfv 198 SGVSNRFSGSKSGSTASLTISGLQAEDEADYYCSSYTTRSTR-VFGGGTK
 CDR L3
 17.scfv 238 VEIK
 3.scfv 246 LTVL
 4.scfv 247 LTVL

FIG. 25

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/US 97/00325

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/715 C07K16/46 C07K19/00 C07K16/28
C12N15/62 A61K39/395 C12N5/10 C12N15/85 G01N33/577
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CELL, vol. 83, no. 7, 29 December 1995, pages 1263-1271, XP000602068 TARTAGLIA L A ET AL: "IDENTIFICATION AND EXPRESSION CLONING OF A LEPTIN RECEPTOR, OB-R" cited in the application	1-4,7,8, 16-23, 25,26, 28-30, 32-37, 39,40, 46,47,58
Y	see the whole document	5,6, 9-15,31
Y	WO 94 05332 A (BERLEX LAB) 17 March 1994 see page 1 - page 10 see page 18; claim 16	5,6
Y	WO 91 01743 A (CEMU BIOTEKNIK AB) 21 February 1991 see page 1, line 1 - page 6, line 30 see page 18; claims	5
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 April 1997

Date of mailing of the international search report

29. 04. 97

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Authorized officer

Macchia, G

INTERNATIONAL SEARCH REPORT

Intern: d Application No

PCT/US 97/00325

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, vol. 88, 1991, pages 10535-10539, XP002029642 ASHKENAZI ET AL.: "Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesin" cited in the application see page 10535	9-12,31
Y	--- US 5 378 808 A (D ANDREA ALAN ET AL) 3 January 1995 see column 3, line 5-19 see column 6, line 62 - column 7, line 34	13-15
P,X	--- WO 96 08510 A (PROGENITOR INC) 21 March 1996	1,2,4, 7-9,13, 14,16, 17, 19-23, 25,26, 28-36, 38,40, 46,47
A	see abstract	37, 41-45, 48-57
P,X	see page 1, line 16 - page 7, line 25 see page 9, line 1 - page 10, line 9 see page 16, line 8 - page 25, line 5 see page 50 - page 53; claims see figure 3B & NATURE MEDICINE, vol. 2, no. 5, 5 May 1996, pages 585-589, XP002019361 CIOFFI ET AL.: "Novel B219/0B receptor isoforms: possible role of leptin in hematopoiesis and reproduction" cited in the application see the whole document	1,2,4,7, 8,16,23, 28-30, 32-35
A		25-27, 36,38-47
A	--- SCIENCE, vol. 271, 5 January 1996, page 29 XP002029643 BARINAGA M: "Obesity: Leptin receptor weighs in" see the whole document	13,14, 16,17,58
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern: 1 Application No

PCT/US 97/00325

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